

**NEW UTILITY PATENT APPLICATION TRANSMITTAL**  
**(Large Entity)***(Only for new nonprovisional applications under 37 CFR 1.53(b))*Docket No.  
1038-765 MISTotal Pages in this Submission  
3**TO THE ASSISTANT COMMISSIONER FOR PATENTS**Box Patent Application  
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

**CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM**

and invented by:

Naveen N. Anand, Brian H. Barber, George A. Cates, Judith E. Caterini and Michel H. Klein

If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:☒ Continuation   ☐ Divisional   ☐ Continuation-in-part (CIP)   of prior application No.: 08/483,576

Enclosed are:

**Application Elements**

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 44 pages and including the following:
  - a. ☒ Descriptive Title of the Invention
  - b. ☒ Cross References to Related Applications *(if applicable)*
  - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
  - d. ☐ Reference to Microfiche Appendix *(if applicable)*
  - e. ☒ Background of the Invention
  - f. ☒ Brief Summary of the Invention
  - g. ☒ Brief Description of the Drawings *(if drawings filed)*
  - h. ☒ Detailed Description
  - i. ☒ Claim(s) as Classified Below
  - j. ☒ Abstract of the Disclosure
3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
  - a. ☐ Formal
  - b. ☒ Informal

Number of Sheets 10

# NEW UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.  
1038-765 MIS

Total Pages in this Submission  
3

## Application Elements (Continued)

4. ☒ Oath or Declaration
- a. ☐ Newly executed (*original or copy*)      ☐ Unexecuted
- b. ☒ Copy from a prior application (37 CFR 1.63(d)) (*for continuation/divisional application only*)
- c. ☒ With Power of Attorney      ☐ Without Power of Attorney
5. ☒ Incorporation By Reference (*usable if Box 4b is checked*)  
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche (*Appendix*)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission (*if applicable, all must be included*)
- a. ☒ Paper Copy
- b. ☒ Computer Readable Copy (*identical to computer copy*)
- c. ☒ Statement Verifying Identical Paper and Computer Readable Copy

## Accompanying Application Parts

8. ☐ Assignment Papers (*cover sheet & document(s)*)
9. ☐ 37 CFR 3.73(B) Statement (*when there is an assignee*)
10. ☐ English Translation Document (*if applicable*)
11. ☒ Information Disclosure Statement/PTO-1449      ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☐ Certificate of Mailing
- ☐ First Class      ☐ Express Mail (*Specify Label No.*): \_\_\_\_\_
15. ☐ Certified Copy of Priority Document(s) (*if foreign priority is claimed*)

# NEW UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.  
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3

## Accompanying Application Parts (Continued)

16. ☐ Additional Enclosures (please identify below):

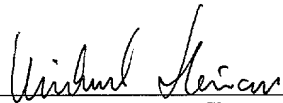
## Fee Calculation and Transmittal

### CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	33	- 20 =	13	x \$22.00	\$286.00
Indep. Claims	9	- 3 =	6	x \$82.00	\$492.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$790.00
OTHER FEE (specify purpose) _____					\$0.00
TOTAL FILING FEE					\$1,568.00

- ☒ A check in the amount of **\$1,568.00** to cover the filing fee is enclosed.
- ☐ The Commissioner is hereby authorized to charge and credit Deposit Account No. \_\_\_\_\_ as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of \_\_\_\_\_ as filing fee.
  - ☐ Credit any overpayment.
  - ☐ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
  - ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: January 12, 1998

  
Signature

M.I. Stewart,  
Registration No. 24,973

CC:

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-765 MIS:as

In re patent application

No.

Applicant: Naveen N. Anand et al

Title: CHIMERIC ANTIBODIES FOR DELIVERY OF  
ANTIGENS TO SELECTED CELLS OF THE  
IMMUNE SYSTEM

Filed:

Group No.

Examiner:

January 13, 1998

PRELIMINARY AMENDMENT

**BY COURIER**

The Commissioner of Patents  
and Trademarks,  
Box Patent Application,  
Washington, D.C. 20231,  
U.S.A.

Dear Sir:

Please amend this application in the following  
manner:

In the Disclosure:

On page 6, line 16, insert "97,202" after "No.";

On page 10, line 11, insert ", comprising panels A  
and B," after "Figure 6"; line 22, insert ",  
comprising panels A and B," after "Figure 9"; line  
23, insert the word "panel" before "A)"; and "B)";  
line 25, insert the word "panel" before "A)"; line  
26, insert "panel" before "B)"; line 27, insert ",  
comprising panels A and B," after "Figure 10"; line  
29, insert "panel" before "A)"; line 30, insert  
"panel" before "B)".

Page 19, line 18, insert "97,202" after "No." and  
insert "June 23, 1995" after "on".

Insert the hard copy of the Sequence Listing after  
the last page of disclosure and immediately  
preceding the claims.

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In the Claims:

Amend claims 1 and 27 as follows:

1. (Amended) A recombinant conjugate antibody molecule, consisting of a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.

27. (Amended) An immunogenic composition, comprising, as an active component thereof, a conjugate antibody molecule consisting of [comprising] a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.

Cancel claims 26 and 29 to 33.

In the Drawings:

It is proposed to Amend Figures 1, 2, 3 and 4 as shown on the enclosed print in red.

REMARKS

In an Advisory Action on the parent application, the Examiner indicated that the Amendment submitted September 24, 1997 would not be entered in the parent filing, on the basis that:

"The proposed amendments to the claims would require an additional art search and application of any art that is found".

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While not agreeing with the Examiner in this regard, this Continuation Application is being submitted with revisions to the claims corresponding to those proposed to be made in the parent application, so that the Examiner can properly consider the same.

In addition, the Examiner indicated in the Advisory Action that the IDS was not considered. The IDS is being re-submitted with this Continuation Application so that the contents may be considered by the Examiner. This new PTO-1449 also includes a listing of the references referred to in the disclosure. Copies of some of the references were provided in the parent filing. The missing copies will follow shortly.

In the Final Action in the parent application, the Examiner rejected claims 1 to 11 under 35 USC 112, second paragraph, as being indefinite, in view of the use of "consisting essentially of" in claim 1. The Examiner's suggestion to employ "consisting of" in claim 1 has been adopted. The same language is used in claim 27. It is submitted that claims 1 to 11 are no longer open to rejection under 35 USC 112, first paragraph.

In the Final Action on the parent application, the Examiner rejected claims 1 to 11 and 27 to 28 under 35 USC 102(a) as being anticipated by Baier et al.

The applicants' claims are directed to a recombinant conjugate antibody molecule which consists of a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on the monoclonal antibody, whereby the conjugate antibody molecule is capable of delivering the antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to the antigen moiety. Applicants claims include immunogenic compositions comprising such molecule.

As previously pointed out, Baier et al teach the provision of a gp-120 peptide and antibody Fab fragments

PTO-1449



11 and 27 to 28 is not anticipated by Baier et al and hence claims 1 to 11, 27 and 28 are not open to rejection under 35 USC 102(a) as being anticipated by Baier et al.

In the Final Action on the parent application, the Examiner rejected claims 1 to 11, 27 and 28 under 35 USC 102(b) as being anticipated by or, in the alternative, under 35 USC 103(a) as being obvious over Barber et al U.S. Patent 4,950,480.

As previously noted in the prosecution of the parent application, the Barber et al reference is acknowledged in the specification, for example, on page 2, line 36 to page 3, line 10. As described therein, biotin-streptavidin based interaction was used to link antibody and antigen to provide a molecule used for targeting the antigen to the antigen-presenting cells. There are inherent disadvantages to the chemical coupling technique employed by Barber et al, such as yield (typically about 20%) and the variability between different preparations. There is no adequate control on the amount of coupled peptide, as well as the exact location of the reaction. Purification is usually required and losses of material can be significant. These disadvantages of the Barber et al system are echoed in the Baier reference in the paragraph bridging pages 2357 and 2358.

The present invention meets the need to produce conjugates of targeting antibodies and antigens of specific reproducible structure in high yields. In the present invention, as defined in claim 1, there is provided a recombinant conjugate antibody molecule which consists of a bivalent monoclonal antibody moiety having heavy and light chains and specific for a surface structure of antigen presenting cells, genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on the monoclonal antibody moiety. This language defines a structure different from that provided by Barber et al.



In the Final Action on the parent application, the Examiner states:

"Examiner agrees that the process by which the antibody-antigen fusion proteins were generated are different. However, product by process is not patentably distinct in a product claim. Applicant is claiming antibody-antigen fusion proteins. The process by which the fusion proteins were generated is irrelevant. In order to demonstrate patentability, applicant must show that the recombinant antigen-antibody fusion proteins have properties which differ from the chemically conjugated fusion proteins." (Emphasis added).

Applicant agrees largely with this analysis. However, applicants claims recite structural limitations which distinguish the claimed product from those described in Barber et al. Applicants claims recite that the monoclonal antibody moiety has been genetically modified to contain the antigen moiety exclusively at at least one preselected site on the monoclonal antibody moiety. This structural limitation is achievable only by recombinant methodology and hence the recitation of a recombinant conjugate antibody molecule also imparts a structural limitation on the molecule which differentiates the structure from that obtained in Barber et al.

The lack of adequate control on the amount of coupled antigen as well as the exact location of the reaction in the prior art of Barber et al ensures that the antigen moiety is not coupled exclusively at at least one preselected site into the monoclonal antibody moiety as required by applicants claims.

The Examiner further stated in the Final Action on the parent file that:

"... applicant argues the disadvantages in the chemical coupling technique. However, as applicant is claiming product claims, the advantages or disadvantages of the different methods used to obtain the product are irrelevant."

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Accordingly, it is submitted that claims 1 to 11 and 27 to 28 are not open to rejection under 35 USC 102(b) as anticipated by, or in the alternative, under 35 USC 103(a) as obvious over U.S. Patent No. 4,950,480.

The Examiner, accordingly, made a third rejection of claims 1 to 11, 27 and 28 based on U.S. Patent No. 4,950,480:

- The necessity to make three rejections of the same claims shows a clear lack of conviction on the part of the Examiner as to the relationship of the claims to the prior art.

"teaches the generation of single chain antibody fusion proteins. As the chains recite a monoclonal antibody moiety, the term would include single chain antibodies."

Applicants have discussed the Examiner's interpretation of the applicant's claim language above with respect to the rejection based on Baier et al. Applicant's claims now specifically recite that the monoclonal antibody has heavy and light



TITLE OF INVENTIONCHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO  
SELECTED CELLS OF THE IMMUNE SYSTEMFIELD OF INVENTION

The present invention is concerned with novel recombinant antibody molecules genetically modified to contain an antigen moiety for the purpose of delivery of the antigen moiety to antigen-presenting cells of the immune system.

BACKGROUND OF INVENTION

Current theories of immunology suggest that, in order to provide a potent antibody response, an antigen must be seen by both B cells, which subsequently develop into the antibody producing cells, and also by helper T-cells, which provide growth and differentiation signals to the antigen specific B-cells. Helper T-cells recognize the antigen on the surface of antigen-presenting cells (APC) in association with Class II major histocompatibility complex (MHC) gene products.

There are significant advantages in using proteins and peptides derived from proteins of infectious organisms as part of subunit vaccines. The search for such suitable subunits constitutes a very active area of both present and past research. Advances in techniques of recombinant DNA manipulations, protein purification, peptide synthesis and cellular immunology have greatly assisted in this endeavour. However, a major stumbling block to the use of such materials as vaccines has been the relatively poor *in-vivo* immunogenicity of most protein subunits and peptides. Generally, the immune response to vaccine preparations is enhanced by the use of adjuvants. However, the only currently licensed adjuvants for use in humans are aluminum hydroxide and aluminum phosphate, collectively termed alum, which is limited in its effectiveness as a potent adjuvant. There is thus a need for new adjuvants with the desired

efficacy and safety profiles.

Several adjuvants, such as Freund's Complete Adjuvant (FCA), syntex and QS21, have been used widely in animals (ref 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). In animals, administration of peptides and protein antigens with these adjuvants, has been shown to result in neutralizing antibodies against a variety of infectious organisms (refs. 3 to 8). A novel way of engaging both the B and T cell components of an immune response has been described, which uses anti-class II monoclonal antibodies (mabs) coupled to antigens to target class II bearing antigen presenting cells (APC's) (refs 9 to 11, also U.S. Patents Nos. 5,194,254 and 4,950,480 assigned to the assignee hereof). Experiments carried out *in-vivo* in rodents and rabbits using this technology, (refs. 9 to 12), have demonstrated convincing proof of enhancement in immunogenicity of antigens, in the absence of conventional adjuvants. Several research groups have used other cell surface markers such as Surface Immunoglobulin (sIg) (ref. 13), Fc  $\gamma$  receptors, CD45 and MHC class I (refs. 14 to 17), to achieve targeting to APC's; however, most of these latter studies involve *in-vitro* experiments and lack animal data. Another group of studies reports the use of antibodies of irrelevant specificity to carry antigen epitopes (refs. 18 to 24). The *in-vivo* studies utilizing such "antigenized antibodies", however, involves the use of conventional adjuvants and some of them require multiple injections for the desired effect (ref. 24).

In previous studies using anti-class II mab as a

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targeting molecule (refs. 9 to 11), biotin-streptavidin based interaction was used to link the antibody and antigen. There are some inherent disadvantages with such chemical coupling techniques, such as yields (about 20%) and also the variability factor between different preparations. There is also no adequate control on the amounts of coupled peptide as well as the exact location of the reaction. Additionally, further purification is usually required and, therefore, losses in material can be significant.

Recently a study reporting *in-vitro* data using anti-human class II Fab-peptide fusions generated by recombinant DNA methodology, has been published (ref. 27). There are several differences between these fusions and the present invention in that the former is an *E. coli* expressed monovalent protein fragment of a divalent whole immunoglobulin molecule and also is an *in-vitro* study. The common problems encountered in bacterial expression systems include expression as inclusion bodies which require solubilization and refolding with extensive product losses. The expression of whole antibody is presently not possible in *E. coli* and, therefore, the monovalent Fab may not have the requisite affinity for *in-vivo* targeting. There are, thus, several advantages in using a whole IgG recombinant system as described herein.

There remains a need, therefore, to produce conjugates of targeting antibodies and antigens of specific reproducible structure in high yields. Such conjugate antibody molecules and nucleic acid molecules encoding the same are useful in immunogenic preparations including vaccines, for protection against disease caused by a selected pathogen and for use as and for the generation of diagnostic reagents and kits.

#### SUMMARY OF INVENTION

The present invention includes novel recombinant

conjugate antibody molecules which have been genetically modified to contain an antigen moiety for delivery of the antigen moiety to antigen-presenting cells of the immune systems.

5       Accordingly, in one aspect of the present invention, there is provided a conjugate antibody molecule, comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells genetically modified to contain at least one  
10 antigen moiety exclusively at at least one preselected site in the monoclonal antibody. The conjugate antibody molecule is capable of delivering the antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to the antigen moiety in the  
15 host.

Genetically modifying the antibody moiety to contain the antigen moiety only at preselected sites ensures that a product with consistent composition and structure is obtained.

20       The antigen presenting cells may be any convenient antigen-presenting cells of the immune system, including class I or class II major histocompatibility expressing cells (MHC), B-cells, T-cells or professional antigen-presenting cells including dendritic cells, and CD4<sup>+</sup>  
25 cells.

The at least one antigen moiety preferably is located at at least one end of at least one of the heavy and light chain of the monoclonal antibody moiety, particularly the C-terminal end of both the heavy and  
30 light chain. The at least one antigen moiety is preferably directly linked with the C-terminal end of both the heavy and light chains of the monoclonal antibody moiety.

One feature of the present invention is the ability  
35 to obtain an enhanced immune response to an antigen without the use of an adjuvant. Accordingly, in one

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embodiment of the invention, the at least one antigenic moiety may comprise an inherently weakly-immunogenic antigen moiety. The at least one antigen moiety may comprise a plurality of antigen moieties, which may be  
5 the same or different. In addition, the at least one antigen moiety may be a peptide having 6 to 100 amino acids and containing at least one epitope.

The novel conjugate antibody molecules provided herein are produced by recombinant procedures which  
10 include the provision of novel nucleic acid molecules and vectors containing the same.

In accordance with another aspect of the present invention, there is provided a nucleic acid molecule comprising a first nucleotide sequence encoding a chain  
15 of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide  
20 sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen. The antigen presenting cells may be any of those described above.

25 The first nucleotide sequence and the second nucleotide sequence are preferably directly linked in a single transcriptional unit under control of the promoter. The third nucleotide sequence preferably is disposed at the 5'-end of the first nucleotide sequence.

30 The present invention further includes vectors comprising the nucleic acid molecules provided herein. In one specific embodiment of this aspect of the invention, this vector may contain a first nucleic acid molecule comprising a first nucleotide sequence encoding  
35 the heavy chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second

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nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody heavy chain and said at least one antigen as a first transcriptional unit, and a second nucleic acid molecule comprising a first nucleotide sequence encoding the light chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody light chain and said at least one antigen as a second transcriptional unit.

One particular vector has the characteristics of plasmid pCMVdhfr.chLCHC (ATCC Accession No. ).

The production of the conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells and at least one antigen moiety in mammalian cells constitutes a further aspect of the invention. Such procedure comprises:

constructing a first nucleic acid molecule containing a first nucleotide sequence encoding a heavy chain of said monoclonal antibody and a second nucleotide sequence encoding at least one antigen,

constructing a second nucleotide acid molecule containing a first nucleotide sequence encoding a light chain of said monoclonal antibody and a second nucleotide sequence encoding said at least one antigen, and

coexpressing said first and second nucleic acid molecules in mammalian cells to form said conjugate antibody molecule.

The coexpression of the first and second nucleic acid molecules includes constructing an expression vector containing the first and second nucleic acid molecules as

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independent transcriptional units, which preferably also contain a promoter operable in mammalian cells to direct the coexpression. The coexpression includes secretion of the conjugate molecule and the conjugate molecules may be  
5 separated from the culture medium and purified, preferably by binding to protein A and selectively eluting the conjugate molecules.

A further aspect of the invention provides an immunogenic composition comprising a conjugate antibody  
10 molecule as provided herein or a nucleic acid molecule as provided herein. The immunogenic composition preferably is formulated as a vaccine for *in vivo* administration to a host to elicit an immune response against disease(s) caused by a pathogen producing the at least one antigen.

15 According to an additional aspect of the invention, there is provided a method of generating an immune response in a host, comprising administering thereto an immunoeffective amount of a immunogenic composition as provided herein.

20 The novel conjugate antibody molecules provided herein also are useful in diagnostic applications. Accordingly, in yet a further aspect of the invention, there is provided a method of determining the presence of a selected antigen in a sample, which comprises:

25 (a) immunizing a host with a conjugate antibody molecule as provided herein, wherein the at least one antigen moiety is said selected antigen to produce antibodies specific to the selected antigen;

(b) isolating the antibodies;

30 (c) contacting the sample with the isolated antibodies to produce complexes comprising any selected antigen in the sample and the selected antigen-specific antibodies; and

(d) determining production of the complexes.

35 The invention further comprises a diagnostic kit for determining the presence of a selected antigen in a

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(g) isolating anti-selected antigen antibodies from the cultures.

# BRIEF DESCRIPTION OF DRAWINGS

The invention is described in more detail herein with reference to the accompanying drawings, in which:

Figure 1A shows the DNA sequence (SEQ ID No: 1) and  
5 derived amino acid sequence (SEQ ID No: 2) of the variable region of murine 44H104 mab light chain. The sequence of the peptide mediating secretion is shown in italicized script.

Figure 1B shows the DNA sequence (SEQ ID No: 3) and  
10 derived amino acid sequence (SEQ ID No: 4) of the variable region of murine 44H104 mab heavy chain. The sequence of the secretory peptide mediating secretion is shown in italicized script.

Figure 2A shows the amino acid sequence (SEQ ID No:  
15 5), in single letter code of peptide CTLB36, and nucleotide sequence encoding the same (SEQ ID No: 6), including two termination codons.

Figure 2B shows a scheme for construction and  
20 assembly of a gene coding for CTLB36 using overlap extension PCR.

Figure 2C shows synthetic polynucleotides CTLB 36.1, CTLB 36.2 and CTLB 36.3 and their sequences (SEQ ID Nos: 7, 8 and 9) used in the scheme of Figure 2B and primers LC.F, HC.F and R and their sequences (SEQ ID Nos: 10, 11  
25 and 12) used in the PCR reaction.

Figure 3A shows a scheme for construction of 44H104 light chain gene using PCR-generated DNA cassettes  $V_L$  and  $C_L$ .

Figure 3B shows the oligonucleotide primers Pr. 1, Pr. 2, Pr. 3 and Pr. 4 (SEQ ID Nos: 13, 14, 15 and 16)  
30 synthesized for PCR reactions to obtain  $V_L$  and  $C_L$  gene cassettes.

Figure 4A shows a scheme for construction of chimeric 44H104 heavy chain gene using PCR-generated  $V_H$  and  $C_H$  DNA cassettes.  
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Figure 4B shows the oligonucleotide primers Pr. 5,

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Pr. 6, Pr. 7 and Pr. 8 (SEQ ID Nos: 17, 18, 19 and 20) synthesized for PCR reactions to obtain V<sub>H</sub> and C<sub>H</sub> gene cassettes.

Figure 5 contains the structures and schemes for construction of pRc/CMV based expression vectors for genes encoding chimeric light and heavy chain fusions with CLTB36. Plasmid pCMV·chLCHC is a tandem co-linear construction with both genes on the same vector. Plasmid pCMVdhfr·chLCHC is a co-linear plasmid with a murine dhfr encoding gene cassette.

Figure 6 shows flow cytometry data demonstrating binding of chimeric antibody conjugates to HUT78 cells. The conjugate is stained with a anti-human Fc specific antibody in panel A and anti-CLTB36 guinea pig serum in panel B.

Figure 7 illustrates anti-CLTB36 IgG titres in macaque sera as measured by ELISA, after immunization and boosting with ch·44H104-CLTB36 conjugates.

Figure 8 illustrates anti-rP24 IgG titres in bleed 1 and 4 of macaques immunized with ch. 44H104-CLTB36 conjugates.

Figure 9 depicts Coomassie blue stained SDS/PAGE gels 7.5% (A) and 10% (B). Gel A was run with samples in non-reducing buffer and gel B in reducing buffer. The bands corresponding to the intact antibody (A) and light and heavy chains (B) are labelled with arrows.

Figure 10 depicts Western blots corresponding to the Coomassie blue stained gels of Figure 9. The bands corresponding to intact antibody conjugate (A) and light and heavy chain conjugates (B) are indicated with arrows. The primary antibody reagent used was anti-CLTB36 guinea pig anti-sera.

#### GENERAL DESCRIPTION OF INVENTION

In the present invention, an antigen, against which it is desired to raise antibodies in a host, generally is conjugated to the C-terminus of both the light and heavy

chains of a monoclonal antibody, which is specific for a particular surface structure of antigen-presenting cells. This arrangement allows for delivery of the antigen to the relevant cells in the immune system upon injection of the conjugate to a host. The monoclonal antibody, therefore, acts as a "vector" or "delivery vehicle" for targeting antigenic determinants to antigen presenting cells, thereby facilitating their recognition by T-helper cells. The antigen presenting cells possess a variety of specific cell surface structures or markers which are targeted by any particular monoclonal antibody. Thus, antigens may be conjugated to a monoclonal antibody specific for any of the surface structures on the antigen presenting cells, including class I and class II major histocompatibility complex (MHC) gene products.

The surface structures on the antigen presenting cells of the immune system which can be recognized and targeted by the monoclonal antibody portion of the immunoconjugates are numerous and the specific surface antigen structure targeted by the monoclonal antibody depends on the specific monoclonal antibody.

The monoclonal antibody may be specific for a gene product of the MHC, and, in particular, may be specific for class I molecules of MHC or for class II molecules of MHC. However, the invention is not limited to such specific surface structures and the conjugates containing the corresponding monoclonal antibodies, but rather, as will be apparent to those skilled in the art, the invention is applicable to any other convenient surface structure of antigen presenting cells which can be recognized and targeted by a specific monoclonal antibody to which an immunogenic molecule is conjugated.

For example, strong adjuvant-independent serological responses to a delivered antigen can be obtained with conjugates formed with dendritic cell-specific monoclonal antibody and CD4<sup>+</sup> cell-specific monoclonal antibody.

5 In the present invention, the monoclonal antibody specific for the target structure is provided in the form of a conjugate with an antigen against which it is desired to elicit an immune response conveniently joined to the C-terminal of the heavy and/or light chains of the monoclonal antibody. While the conjugate antibody molecules are illustrated by such C-terminal connection, the antigen moiety alternatively may be inserted within the light and heavy chains of the antibody and such 10 insertions may establish a particular constrained conformation of the antigen and, in particular, epitopes, within the known structural framework of an antibody molecule. Such conjugate antibody molecules may be conveniently produced by genetic modification of a gene 15 encoding the heavy and light chains of the antibody to contain a gene encoding one or more antigen(s) and coexpressing the resulting nucleic acid molecules.

20 The invention is particularly useful for antigen molecules which normally possess a weakly-immunogenic response, since that the response is potentiated by the present invention. The antigen molecule may be in the form of a peptide or protein, as discussed above, but is not limited to such materials.

25 The present invention is applicable to any antigen which it is desired to target to antigen presenting cells using the monoclonal antibody. The antigen may be a protein or a peptide of 6 to 100 amino acids comprising an amino acid sequence of an epitope. Representative organisms from which the antigen may be derived include 30 influenza viruses, parainfluenza viruses, respiratory viruses, measles viruses, mumps viruses, human immunodeficiency viruses, polio viruses, rubella viruses, herpes simplex viruses type 1 and 2, hepatitis viruses types A, B and C, yellow fever viruses, smallpox viruses, 35 rabies viruses, vaccinia viruses, reo viruses, rhinoviruses, Coxsackie viruses, Echoviruses,

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(CLTB36) gene was constructed using overlap extension PCR. The genes were cloned into an expression vector, transfected into YB2/0 cells and gene amplification carried out using a murine dhfr cassette cloned into the same expression plasmid. Several clones secreting adequate levels of the properly folded and assembled product were identified. The antigen fusions at the C-terminus of the light and heavy chain do not affect the proper assembly of the antibody (see Figure 9) which also maintains its binding specificity (see Figure 6).

As described in U.S. Patents Nos. 4,950,480 and 5,194,254, coupling a weak antigen to the specific monoclonal antibody results in an enhancement of the immunogenicity of such antigen, while avoiding the use of adjuvants and hence represents a much safer immunization procedure which can utilize materials from which only a weak immune response is achieved. Examples of such materials are small peptides which are epitopes of larger proteins or are protein subunits of a pathogen.

For human use, it is desirable that the antibody be modified to produce a mouse/human chimeric antibody, since extensive anti-murine monoclonal antibody responses would be generated by administration of a murine antibody to humans. Since the invention is broadly applicable to any species, it is desirable that, when a conjugate antibody molecule is administered to a specific species, the murine antibody sequences be replaced by corresponding sequences from the specific species in an analogous manner to that described herein for the mouse/human chimeric antibodies.

The experimental data presented herein and detailed in the Examples below demonstrate the ability of a mouse/human chimeric antibody, which targets antigen presenting cells (APC's) of the immune system via their surface MHC class II receptors, to enhance the immune response to a peptide antigen conjugated to the C

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terminus of both the light and heavy chains. Such a conjugate can be produced conveniently, as detailed in the Examples, using recombinant DNA methodology, namely by assembling the genes encoding both the light and heavy chains with CLTB36 or other antigen of interest in a suitable expression vector. The vector pRC/CMV was selected as the basic expression plasmid in the experimental work performed herein, since it uses the powerful and broad host range immediate early CMV promoter to drive transcription. The final construct was designed to contain both light and heavy chain genes on the same vector as independent transcriptional units. The murine dhfr gene encoding cassette was also incorporated in this specific vector to provide a suitable means of gene amplification. This expression vector was electroporated into rat myeloma YB2/0 cells. Cell lines expressing recombinant antibody were established. Using the amplification procedure outlined in the Examples below and reported in the literature (ref. 32) stable cell lines secreting viable amounts of recombinant antibody conjugate (approximately 30  $\mu$ g/ml) established relatively quickly (in about 4 months). The recombinant chimeric conjugate is assembled correctly and has the same specificity as the parent mab 44H104.

The recombinant conjugate, when administered to macaques without an extrinsic adjuvant (e.g. alum or syntex), elicits good priming immune response, as measured by IgG titres to the peptide antigen on the conjugate. This response is also directed towards the native antigen as measured by recombinant P24 reactivity. The priming response fades after a while but was boosted in two out of three animals by another dose of the chimeric mab conjugate in PBS.

The experimental data presented herein and detailed below, demonstrates the enhancement of immune response to a peptide antigen in the absence of conventional

adjuvants, by coupling to an anti-class II chimeric antibody, the conjugate being generated by recombinant means. The conjugate can be obtained in large amounts by expression in cells, such as YB2/O cells.

5        It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of diseases produced by selected pathogens. A further non-limiting discussion of such  
10        uses is further presented below.

#### 1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the conjugate antibody molecules as disclosed herein. The vaccine elicits an  
15        immune response in a subject which produces antibodies including anti-antigen moiety antibodies. Should the vaccinated subject be challenged by a pathogen that produces the antigen moiety, the antibodies bind to and inactivate the pathogen.

20        Immunogenic compositions including vaccines may be prepared as injectables, as liquid solutions or emulsions. The conjugate antibody molecules may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include,  
25        water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, or pH buffering agents. Immunogenic compositions and vaccines may be administered  
30        parenterally, by injection subcutaneously or intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the  
35        immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral

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(intragastic) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the conjugate antibody molecules. The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune-system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms or milligrams of the conjugate antibody molecules. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of antigen in an immunogenic composition according to the invention is in general about 1 to 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for

example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

The nucleic acid molecules encoding the conjugate antibody molecules of the present invention may also be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization. Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al, 1993 (ref. 33).

## 2. Immunoassays

The conjugate antibody molecules of the present invention are useful as immunogens for the generation of anti-antigen moiety antibodies (including monoclonal antibodies for use in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the anti-antigen moiety antibodies are immobilized onto a selected surface, for example, a surface capable of binding proteins such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of test sample onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to

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## Biological Deposits

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## EXAMPLES

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employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Enzymes and reagents commonly used in standard recombinant DNA technology manipulations were purchased from Böehringer Mannheim, New England Biolabs, Gibco/BRL and Pharmacia. Many specific reactions were performed using Reagent Kits which were purchased from several sources indicated in the specific Examples below. Antibody reagents for ELISAs were purchased from Caltag unless otherwise indicated. Plasmid vectors were purchased from Gibco/BRL or Invitrogen. Polymerase Chain Reaction (PCR) was performed using protocols and kits (Gene Amp PCR System) supplied by Perkin Elmer Cetus. The Thermal cycler used in PCR reactions was purchased from Perkin Elmer Cetus.

The synthesis of oligonucleotides was carried out using an Applied Biosystems 380B DNA synthesizer. The synthesized oligonucleotides were purified on OPC cartridges supplied by Applied Biosystems following the manufacturers protocols. DNA sequencing was performed on an automated DNA sequencer (370A; Applied Biosystems), using the dideoxy terminator chemistry and reagents supplied by the manufacturer.

Example 1:

This Example illustrates cDNA synthesis and sequence determination.

The hybridoma cell line 44H104 secreting murine anti-human class II mab (IgG2aK) was grown in RPMI medium, (Gibco-BRL) supplemented with glutamine (2mM), penicillin (50ug/ml) and streptomycin (50U/ml) and containing 10% FBS. Cells ( $10^6$ ) were harvested and mRNA isolated using a 'Fast Track mRNA Isolation' kit (Invitrogen). First and second-strand cDNA was prepared using the 'cDNA synthesis Plus' kit (Amersham) and protocols supplied by the manufacturer. The cDNA generated in this step was cloned into  $\lambda$ gt10 using the

'cDNA Cloning System- $\lambda$ gt10' kit (Amersham) to generate a lamda phage cDNA library. A cDNA library from the mRNA of mab 44H104 secreting cell line was made in lambda phage. Phage clones containing genes encoding the light and heavy chains were identified. PCR reactions were also performed on the cDNA (50 ng) using primers and conditions used by Winter and colleagues (Ref 28). The amplified products corresponding to  $V_L$  and  $V_H$  of 44H104 were labelled with  $P^{32}$  using the 'Random priming system I' kit (New England Biolabs) and used as probes to isolate phage clones containing inserts encoding the light and heavy chain genes.

The inserts were excised and cloned into the multilinker region of pUC18. These were sequenced and the nucleotide sequence of both  $V_L$  and  $V_H$  are displayed in Figure 1 and 1B respectively (SEQ ID Nos: 1 and 2). The italicised sequences in this figure are the sequences of the signal peptide which precede the mature sequences of the light and heavy chains. Most standard manipulations were performed using well described protocols (ref. 29).  
Example 2:

This Example illustrates construction of a gene encoding peptide antigen CTLB36.

Antigen peptide CLTB36 (Figure 2A, SEQ ID No: 5), which consists of a tandemly linked T and B cell epitope, derived from the sequence of MN strain of HIV, was constructed by PCR using the overlap extension method (illustrated in Figure 2B). The nucleic acid sequence encoding CLTB36 was deduced from the amino acid sequence of the peptide antigen (Figure 2A, SEQ ID No: 6). The procedure consists of synthesizing three oligonucleotides (CLTB36.1, CLTB36.2 and CLTB36.3; Figure 2C, SEQ ID Nos: 7, 8 and 9) which span the entire gene. The oligonucleotide CLTB36.1 was designed to have 16 bases at the 3' end, complementary (overlap) to the 5' end of CLTB36.2, which in turn has a 16 base overlap at its 3'



end with corresponding 5' nucleotides of oligonucleotide CLTB36.3. Polynucleotide primers designated as PrLC.F and PrHC.F were also synthesized; these were designed to overlap with the 5' of the gene coding for CLTB36 and provide a *Bam*HI site for incorporation into the light chain gene or a *Kpn* I site for fusion with the heavy chain gene (Figure 2C, SEQ ID Nos: 10 and 11). The last primer (Pr.R) is the 'back' primer and has homology to the 3' end of the CLTB36 gene and was designed to provide a *Hind* III site for cloning into the expression plasmid (Figure 2C, SEQ ID No: 12).

The oligonucleotides CLTB36.1, CLTB36.2, and CLTB36.3 were mixed together (30 pm each) in PCR reaction buffer heated up to 90°C and slowly annealed at about 45°C. Subsequently the volume was made up to 100  $\mu$ l by adequate additions of buffer, dNTP's primers (PrLC.F and PrR for light chain antigen; PrHC.F and Pr.R for heavy chain antigen; 100 pmol each) using material and protocols from a Gene Amp PCR kit and a PCR reaction was performed. The aqueous phase of the reaction mixture was removed to another tube and an aliquot (5  $\mu$ l) was ligated into pCRII vector and cloned using a 'TA cloning kit' (Invitrogen). The insert was sequenced and clones containing the correct sequence excisable by the correct combination of restriction sites were established.

#### Example 3:

This Example illustrates assembly of the gene encoding the chimeric light chain of 44H104 conjugated to mab CTLB36.

The  $V_L$  of 44H104 and its natural signal sequence was obtained by PCR amplification using pUC18-LC (pUC18 vector containing a light chain encoding cDNA insert) as a template. The two primers used in the reaction (Pr 1 and 2; Figure 3B, SEQ ID Nos: 13, 14) were designed to (a) incorporate a *Hind* III restriction site followed by a Kozak consensus sequence (CCGCC; ref. 3) at the 5' of

the amplified product and (b) incorporate an *Xho* I restriction site at the junction of  $V_L$  and  $C_L$  by creating a silent mutation. The PCR reactions were carried out using 50 ng of template, 100 pmol each of the primers in a 100  $\mu$ l volume using buffers, dNTP's and enzyme supplied in the GeneAmp kit. The cycling parameters were: 95°C for 1 min., 55°C for 1 min. followed by 72°C for 2 min., for a total of 25 cycles. An aqueous aliquot of the final reaction mixture was analyzed on a 10% agarose gel and another aliquot (5  $\mu$ l) was ligated into pCRII vector supplied in the 'TA Cloning' kit (Invitrogen). The ligation reaction was used to transform competent *E. coli* cells plated out on X-Gal agar plates containing ampicillin. Plasmid was isolated from several colonies bearing a white phenotype and sequenced. Approximately one in three clones were found to have the correct sequence.

The human light chain constant (Kappa) gene required for the construct encoding chimeric 44H104 light chain was also obtained by PCR amplification. The template was a plasmid pUC19-k containing an insert coding for the human kappa gene. The primers used in the PCR reaction (pr. 3 and 4; Figure 3B, SEQ ID Nos: 15 and 16) were designed to incorporate an *Xho* I restriction site at the 5' end of the cassette suitable for ligation with the  $V_L$  gene obtained above. These primers also incorporate a *Bam*HI site at the 3' end to enable ligation to the antigen-CLTB36 gene. The PCR reaction was carried out in the same way as described above for  $V_L$  gene of 44H104, cloned into pCRII vector and clones carrying inserts identified and sequenced. Two clones having the correct sequence were set aside for further work.

The pCRII vector containing  $V_L$  gene insert was digested with a combination of *Hind* III and *Xho* I restriction endonucleases and the 400 bp insert isolated. Similarly polynucleotide fragments encoding the human

Kappa gene and CLTB36 were excised out of pCRII cloning vectors using digestion with combinations of *Xho* I/*Bam*H I and *Bam*H I/*Hind* III respectively. All three fragments were mixed (10-20 ng each) and ligated into an aliquot of *Hind* III digested expression plasmid pRC/CMV (Invitrogen) using standard protocols. The ligation reaction was used to transform competent *E. coli* TG1 cells and recombinants analyzed for inserts. The orientation of the insert was ascertained by restriction enzyme digest patterns and confirmed by DNA sequencing. This plasmid was designated as pCMV.chLC (Figure 5).

Example 4:

This Example illustrates assembly of a gene encoding the chimeric heavy chain of 44H104 mab conjugated with CTLB36.

The gene for the chimeric heavy chain conjugated to CLTB36 was constructed from gene cassettes, generated in a manner similar to what has been described for the light chain in Example 3. The detailed scheme and sequences of the oligonucleotide primers are shown in Figure 4. Synthetic oligonucleotide primers 5 and 6 (SEQ ID Nos: 17, 18) were used in generating the  $V_H$  gene from a plasmid template (pUC18) containing a cDNA insert encoding the heavy chain of mab 44H104. The primers were designed to incorporate a 5' *Hind* III restriction site, a kozak sequence and a silent mutation at the 3' ( $V_H$ - $C_H$  junction) resulting in a *Spe* I site for ligation to the constant domain gene. The PCR product was cloned into pCRII vector and the nucleotide sequence integrity of the insert confirmed. The human constant domain ( $C \gamma 1$ ) gene was obtained by the amplification of the insert encoding this in plasmid pUC19-G1 using PCR primers 7 and 8 (SEQ ID Nos: 19, 20). As with primers Pr. 5 and Pr. 6, the primers were designed to engineer a 5' *Spe* I site for ligation to the  $V_H$  gene and a *Kpn* I recognition site fusion to the antigen gene. The PCR products were cloned

into pCRII as before, and correct clones identified by DNA sequencing.

The gene cassettes encoding  $V_H$ , human C $\gamma$ 1 and CLTB36 were obtained from sequences inserted into pCRII plasmid by digestion with combinations of *Hind* III/*Spe* I, *Spe* I/*Kpn* I and *Kpn* I/*Hind* III restriction enzymes respectively. The correct DNA fragments were isolated from agarose gels, mixed and ligated into *Hind* III digested pRC/CMV plasmid. These were used to transform competent *E.coli* cells and plasmid isolated from selected colonies. The plasmid was checked for inserts encoding the chimeric heavy chain-CLTB36 conjugate. The orientation of the gene with respect to the rest of the expression plasmid was established using restriction enzyme digestion patterns. The insert was also sequenced, the expression plasmid was designated pCMV.chHC (Figure 5).

#### Example 5

This Example illustrates construction of expression plasmids.

The DNA sequences encoding the CLTB36 fusions with chimeric light and heavy chains were assembled in pRC/CMV (Invitrogen) to give plasmids pCMV.chLC and pCMV.chHC respectively (Figure 5), as described in Examples 3 and 4. A single expression vector containing the genes for both light and heavy chains as distinct transcription units each under their own CMV promoter was constructed (the scheme is shown in Figure 5). pCMV.chHC plasmid was digested with *Nru* I and *Dra* III and a 2.8 kb DNA fragment isolated on a 0.8 % agarose gel. The DNA fragment was blunt ended following a standard protocol (ref. 30) and using dNTP's and DNA polymerase (Klenow). The resulting DNA fragment was then ligated into plasmid pCMV.chLC linearized by digestion with *Nru* I restriction enzyme and the resulting co-linear vector designated as pCMV.chLCHC. The orientation and general structure of the plasmid is

as shown in Figure 5 and was confirmed by extensive restriction enzyme digestion analysis.

Expression plasmid pCMVdhfr.chLCHC was constructed by inserting a blunt ended 1.9 kb *Pvu* II/*Bam*H I fragment from plasmid pSV2.dhfr (ref. 31), into the *Bgl* II restriction site of vector pCMV.chLCHC. This DNA fragment encodes a murine dihydrofolate reductase gene under the control of a SV40 promoter and terminating in a SV40 poly A. The orientation of the insert was confirmed by restriction digest analysis and is as shown for pCMVdhfr.chLCHC in Figure 5. This plasmid was isolated from transformed TG1 cells by banding on cesium chloride (ref. 30) and used in transfection experiments.

Example 6:

This Example illustrates the expression of chimeric 44H104-CLTB36 conjugates.

Initial expression was attempted by co-transfecting plasmids pCMV. chLC and pCMV.chHC prepared as described in Examples 3 and 4, into non-Ig secreting murine SP2/0 myeloma cells by electroporation. The SP2/0 cells were grown to mid log phase and then harvested;  $1 \times 10^7$  cells were washed with cold PBS, centrifuged (4-5xg, for 5 min) and resuspended in 0.5 ml of PBS. Plasmid DNA linearized with *Bgl* II enzyme (10  $\mu$ g of each plasmid) was added to the cell suspension and the mixture incubated on ice for 10 minutes. The suspension was transferred to a cold 0.4 cm electroporation cuvette and subjected to an electrical pulse at a setting of 700V and capacitance of 25  $\mu$ F in a 'Gene Pulsar' electroporator (Biorad). The mixture was further incubated in ice (5 min.) and then left in supplemented RPMI (with 10% FBS) for 48 hours. Subsequently the cells were plated out in selective media consisting of RPMI medium supplemented with 10% FBS and 600  $\mu$ g/ml of G418 (Sigma) in 96 well plates ( $1 \times 10^4$  cells per well). The media was replaced every three days and after 2 weeks, wells displaying cell growth were

checked for recombinant antibody secretion in supernatants by ELISA. Several pools/wells were selected and cloned by dilution cloning method (ref. 30) and again checked for ch. mab secretion. A few selected clones were  
5 expanded and stored as stocks with DMSO in liquid nitrogen. The expression plasmid pCMV.chLCHC was also used to transfect SP2/0 cells. The methodology of electroporation and establishment of cloned cell lines secreting chimeric mab-CLTB36 conjugates are as described  
10 above. The overall yield was, however, quite low.

The expression plasmid pCMVdhfr.chLCHC, prepared as described in Example 5, was transfected into YB2/0 rat myeloma cells (ATCC CRL 1662) following the protocols detailed by Shitara et al. (ref. 32). Essentially YB2/0  
15 cells were grown in supplemented RPMI (containing 2mM glutamine; penicillin 50ug/ml and streptomycin 50 U/ml) containing 10% FBS. Aliquots of  $1 \times 10^7$  cells were collected, washed in PBS and taken up in 250  $\mu$ l of PBS. These were mixed with non-linearized plasmid  
20 pCMVdhfr.chLCHC (10  $\mu$ g) and electroporated at 200V, 250  $\mu$ F capacitance in a Gene Pulsar electroporator (Bio Rad). The cells were then treated exactly like the electroporated SP2/0 cells described above and after 48 hours in non selective media were plated into ten 96-well  
25 plates in supplemented RPMI containing 600  $\mu$ g/ml of G418. The media from wells displaying cell growth were analyzed for recombinant antibody and pools secreting the desired product identified. Some selected pools were transferred to 6 well plates and the media was replaced with  
30 supplemented RPMI containing 10% FBS, 600  $\mu$ g/ml G418 and 50 nM of methotrexate (Sigma). The pools were adapted to this concentration of methotrexate (MTX) and then the level was increased to 100 nM. Subsequently the concentration of MTX was increased to 200 nM, then 500  
35 nM, 1000 nM and finally 1500 nM. The cells were adapted to each of these levels through several passages and

finally cloned by limiting dilution. Several clones secreting recombinant products from 3 to 30  $\mu\text{g/ml}$  of spent culture medium (after protein A purification) were obtained and were used to obtain the chimeric mab in quantities large enough to permit experimentation in animals.

96 well microtitre plates (Maxisorp Immuno; Nunc) were coated with a Goat anti-human-kappa light chain antibody fragment. The plates were washed in PBST (PBS containing 0.05% Tween 20), blocked with 0.1% casein in PBST, and incubated with aliquots (100  $\mu\text{l}$ ) of culture supernatants. A human myeloma IgG1K (Pharmingen) was used as a positive control. After washing, the plates were incubated with a goat anti-human IgG (Fc specific) F(ab')<sub>2</sub> conjugated to alkaline phosphatase. The un-bound conjugate was washed out and substrate pNPP (Gibco/BRL) was added to the wells in phosphatase buffer. After about 15 min and the colour development measured in a Dynatech MR5000 ELISA plate reader at a setting of 405-410 nm.

#### 20 Example 7:

This Example describes the isolation and purification of ch 44H104-CLTB36 conjugates.

Clones identified as high producers of conjugate in Example 6, exclusively from the pCMVdhfr.chLCHC transfection of YB2/0 cells and subsequent gene amplification experiments, were scaled up in supplemented RPMI containing G418 (600  $\mu\text{g/ml}$ ), methotrexate (1 $\mu\text{M}$ ) and 10% ultra low IgG FBS (from Gibco/BRL). The cells were allowed to grow in T-flasks until approximately half of them were dead (approximately 1 week). The culture was centrifuged and the supernatant collected. The spent media was stored at 4°C with 0.1% sodium azide to prevent microbial growth.

The ch 44H104-CLTB36 conjugates in the supernatant were isolated by Protein A purification. The supernatant was passed through a Protein A-HyperD column (Sepracor).

The column was washed and the bound material eluted with 0.2M glycine (pH 2.8); the fractions containing bound material were neutralized in 1.0M Tris (pH 8.0) and pooled. The fractions were dialyzed against PBS and finally concentrated on Amicon micro-concentrators. The protein content of the pooled, dialyzed and concentrated material was determined using a Standard Protein Assay Kit (Biorad Laboratories). The conjugate was stored at 4°C in PBS.

To remove any high molecular weight aggregates, the Protein A purified material was further fractionated on a Sephacryl S-300 (HR; 9.5 x 90 cm) hplc column. The column was equilibrated with PBS and the sample applied in 2ml aliquots. The column was run at a flow rate of 1 ml/min in PBS and the effluent monitored at 280 nm. The void volume peak (consisting of any aggregates) was collected separately from the peak corresponding to the non-aggregated material. The latter fractions were pooled and concentrated using a YM-10 ultra filtration membrane (Amicon).

#### Example 8:

This Example describes characterization of ch mab 44H104-CLTB36 conjugate.

The conjugate produced following the procedure of Example 7 was assembled as a covalently linked dimer of heterodimers comprised of light and heavy chains. This was demonstrated by SDS/PAGE electrophoresis on 7.5 and 10% gels, running samples in non-reducing and reducing buffer respectively (see Figure 9). The presence of CLTB36 peptide on the conjugates was determined by Western blotting using anti-CLTB36 guinea pig serum generated in house. The second antibody used in these experiments was a Goat anti-guinea pig IgG-alkaline phosphatase conjugate (Jackson Laboratories) (see Figure 10).

The conjugate was also analyzed for binding to class



II molecules on HUT78 cells by Flow Cytometry using binding of recombinant conjugate to HUT78 cells. HUT78 cells (Human MHC class II expressing T cell lymphoma cells) were grown in supplemented RPMI containing 10% FBS. An aliquot of cells ( $1 \times 10^6$  cells/tube) was distributed into 15 ml conical centrifuge tubes and washed with 2 ml of binding buffer (PBS containing 0.1% BSA and 0.1%  $\text{NaN}_3$ ). The cells were collected after centrifugation ( $400 \times g$  for 5 min at  $4^\circ\text{C}$ ) and the pellet resuspended in binding buffer containing different concentrations of recombinant antibody conjugate (Figure 8). The tubes were incubated on ice for 60 minutes with occasional shaking and then washed twice with chilled ( $4^\circ\text{C}$ ) washing buffer (2 ml). The cells were suspended in 100  $\mu\text{l}$  of a 1:20 dilution of fluorescein isothiocyanate-conjugated goat anti-human IgG (Fc specific; Sigma Chemical Co.) and incubated further on ice for 30 minutes with occasional agitation. The cells were washed in binding buffer (2X) and subsequently once in PBS containing 0.1% sodium azide ( $\text{NaN}_3$ ). The cells were finally suspended in an aliquot of 1% paraformaldehyde in PBS (0.5 ml) and analyzed in the EPIC V flowcytometer (Coulter, Harpendon UK).

The recombinant conjugate was also analyzed for the presence of CLTB36 peptide by the same technique. For this analysis, the anti-human conjugate in the above protocol was substituted with anti-CLTB36 guinea pig serum generated in house. This step was followed by 100  $\mu\text{l}$  of 1:50 dilution of biotin-conjugated mouse IgG2b anti-guinea pig mab (sigma) for 30 minutes and finally with 100  $\mu\text{l}$  of a 1:5 dilution of a streptavidin-phycoerythrin conjugate (Becton Dickinson; 30 min). Cells were washed as before and fixed with 1% paraformaldehyde in PBS and analyzed in the flowcytometer. Negative controls, consisting of cells treated as described above but without the incubation

step with recombinant mab conjugate, were used in both assays.

The results obtained are shown in Figure 6. This analysis demonstrates the availability on the surface of cells of the peptide for binding to antibody.

Example 9:

This Example describes immunization of macaques with ch 44H104-CLTB36 conjugates.

The immunogen (mab conjugate), prepared as in Example 7, was concentrated and filtered through a 0.22  $\mu$ M filter. The protein concentration of this was estimated to be about 0.58 mg/ml in PBS.

Three cynomologous macaques were selected and serum samples from them these were screened for adventitious viral agents, such as SA8, HSV-1, HSV-2, V. Zoster, Chimp CMV, EBV, SRV-1, SRV-2, SRV-5, SIV, STLV-1, and B virus. The selected macaques (#197, 198 and 200) were bled and injected intramuscularly with 1.5 ml of PBS (containing 800  $\mu$ g of protein, equivalent to 80  $\mu$ g of peptide). The schedule set forth in the following Table 1 was established.

TABLE 1

Week	Procedure
0	Pre-bleed  Primary injection (0.8 mg of conjugate each)
2	Bleed 1
4	Bleed 2
6	Bleed 3  Boost 1 (0.8 $\mu$ g of conjugate each)
8	Bleed 4
10	Bleed 5

The serum samples from the pre-bleed and Bleeds 1 to 5 were screened for anti-CLTB36 reactivity.

96 well microtitre plates (Polystyrene; Dynatech Labs) were coated with 10  $\mu\text{g/ml}$  of CLTB36 in Carbonate-Bicarbonate buffer (0.05M; pH 9.6). The wells were blocked with 5% skim milk in PBS and subsequently washed in PBS-Tween 20 (0.05%). The serum samples were diluted serially (in 1% skim milk with 0.05% Tween 20) into the wells and incubated at 37°C for 2 hours. The plates were washed and incubated with Goat anti-monkey IgG F(ab')<sub>2</sub> conjugated to Horse Radish Peroxidase (Cappel Laboratories). The excess conjugate was washed off and the colorimetric substrate TMB/H<sub>2</sub>O<sub>2</sub> (ADI) added. The reaction was stopped after 5 min and absorbance measured at 450 and 540 nm in an ELISA Plate reader (EL 310; Biotech Instruments).

The protocol and reagents for an ELISA for P24 reactivity were as described for CLTB36 above; the difference being that the 96 well microtitre plates were coated with recombinant P24 (Dupont) at 1  $\mu\text{g/ml}$  concentration in Carbonate-Bicarbonate buffer.

The IgG titres in different bleeds reactive against CLTB36 and measured by ELISA, are shown in Figure 7. As may be seen, good priming responses were elicited by the recombinant targeting conjugate in PBS, in all three animals (up to about 1 in 25,000 in one animal). The observed ELISA titres diminish after 4 and 6 weeks and then increase again after a boosting dose of the immunogen. The boost in IgG titres was especially prominent in two animals out of the three, the third for unexplained reasons did not boost after such a promising primary response.

The pre-bleed monkey sera and Bleed 1 and 4 (2 weeks post priming and 2 weeks post boosting respectively) were also evaluated for IgG responses against recombinant P24 (CLTB36 has an epitope derived from this portion of HIV

protein). Detectable P24 titres were measured in all three animals and are presented in Figure 8.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention  
5 provides novel recombinantly-produced molecules  
containing an antigen moiety and a monoclonal antibody  
moiety, wherein the monoclonal antibody moiety is  
specific for a determinant expressed on antigen-  
presenting cells of a host, procedures for assembly of  
10 such molecules, nucleic acid molecules encoding such  
molecules and immunizing procedures using such molecules,  
whereby an enhanced immune response to the antigen  
moiety is achieved in the absence of adjuvants.  
Modifications are possible within the scope of this  
15 invention.

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## CLAIMS

What we claim is:

1. A recombinant conjugate antibody molecule, comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host.
2. The molecule of claim 1 wherein said antigen presenting cells are selected from the group consisting of class I major histocompatibility expressing cells, class II major histocompatibility expressing cells, dendritic cells and CD4<sup>+</sup> cells.
3. The molecule of claim 1 wherein said at least one antigen moiety is located at at least one end of at least one of the heavy and light chains of said monoclonal antibody moiety.
4. The molecule of claim 3 wherein said at least one antigen moiety is located at the C-terminal end of said at least one of the heavy and light chains of said monoclonal antibody moiety.
5. The molecule of claim 4 wherein said at least one antigen moiety is located at the C-terminal end of both said heavy and light chains of said monoclonal antibody moiety.
6. The molecule of claim 5 wherein said at least one antigen moiety is directly linked to the C-terminal end of both said heavy and light chains of said monoclonal antibody moiety.
7. The molecule of claim 6 wherein said at least one antigen moiety is an inherently weakly-immunogenic antigen moiety.

8. The molecule of claim 6 wherein said at least one antigen moiety comprises a plurality of antigen moieties.

9. The molecule of claim 8 wherein said plurality of antigen moieties is a plurality of a single antigen moiety.

10. The molecule of claim 8 wherein said plurality of antigen moieties is a plurality of different antigenic moieties.

11. The molecule of claim 7 wherein said at least one antigen moiety is a peptide having from 6 to 100 amino acids and containing at least one epitope.

12. A nucleic acid molecule, comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

13. The nucleic acid molecule of claim 12 wherein said encoded chain is the heavy chain of the monoclonal antibody.

14. The nucleic acid molecule of claim 12 wherein said encoded chain is the light chain of the monoclonal antibody.

15. The nucleic acid molecule of claim 12 wherein antigen presenting cells are selected from the group consisting of class I major histocompatibility expressing cells, class II major histocompatibility expressing cells, dendritic cells and CD4<sup>+</sup> cells.

16. The nucleic acid molecule of claim 12 wherein said first nucleotide sequence and said second nucleotide sequence are directly linked in a single transcriptional unit under control of said promoter.

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17. The nucleic acid molecule of claim 16 wherein said third nucleotide sequence is disposed at the 5' end of said first nucleotide sequence.

18. A vector comprising the nucleic acid molecule of claim 12.

19. The vector of claim 18 containing a first nucleic acid molecule comprising a first nucleotide sequence encoding the heavy chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody heavy chain and said at least one antigen as a first transcriptional unit, and a second nucleic acid molecule comprising a first nucleotide sequence encoding the light chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody light chain and said at least one antigen as a second transcriptional unit.

20. The vector of claim 19 having the characteristic properties of plasmid pCMVdhfr.chLCHC.

21. A method of making a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen-presenting cells and at least one antigen moiety, which comprises:

constructing a first nucleic acid molecule containing a first nucleotide sequence encoding a heavy chain of said monoclonal antibody and a second nucleotide sequence encoding at least one antigen,

constructing a second nucleotide acid molecule containing a first nucleotide sequence encoding a light chain of said monoclonal antibody and a second nucleotide

sequence encoding said at least one antigen, and

coexpressing said first and second nucleic acid molecules in mammalian cells to form said conjugate antibody molecule.

22. The method of claim 21 wherein said coexpression of said first and second nucleic acid molecules includes constructing an expression vector containing said first and second nucleic acid molecules as independent transcriptional units.

23. The method of claim 22 wherein each said independent transcriptional unit includes a promoter operable in mammalian cells to direct said coexpression.

24. The method of claim 23 wherein said expression vector has the characteristic properties of plasmid pCMVdhfr.chLCHC.

25. The method of claim 23 wherein said coexpression includes secretion of said conjugate antibody molecule and further separating and purifying said conjugate antibody molecule.

26. The method of claim 25 wherein said purification comprises binding of the conjugate antibody molecule to protein A and selective elution of said conjugate antibody molecule from protein A.

27. An immunogenic composition, comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group

consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

28. The immunogenic composition of claim 27 formulated as a vaccine for *in vivo* administration to a host to confer protection against disease caused by a pathogen producing said at least one antigen.

29. A method of generating an immune response in a host, comprising administering thereto an immuno-effective amount of an immunogenic composition comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen.

30. A method of determining the presence of a selected antigen in a sample, which comprises:

(a) immunizing a host with a conjugate antibody molecule, comprising a monoclonal antibody moiety

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specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host, wherein said at least one antigen moiety is said selected antigen to produce antibodies specific to said selected antigen;

(b) isolating said antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any selected antigen in the sample and said selected antigen-specific antibodies; and

(d) determining production of the complexes.

31. A diagnostic kit for determining the presence of a selected antigen in a sample, comprising:

(a) a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host, wherein the at least one antigen moiety is said selected antigen;

(b) means for detecting the production of complexes comprising any selected antigen in the sample and selected antigen-specific antibodies to said selected antigen; and

(c) means for determining production of the complexes.

32. A method for producing antibodies specific for a

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selected antigen comprising:

- (a) immunizing a host with an effective amount of an immunogenic composition comprising a conjugate antibody molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to said antigen moiety in the host or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen, wherein said at least one antigen is the selected antigen to produce antibodies specific for the selected antigen; and
- (b) isolating the antibodies from the host.

33. A method of producing monoclonal antibodies specific for a selected antigen comprising:

- (a) administering an immunogenic composition comprising a conjugate molecule comprising a monoclonal antibody moiety specific for a surface structure of antigen presenting cells genetically modified to contain at least one antigen moiety exclusively at at least one preselected site on said monoclonal antibody moiety, whereby said conjugate antibody molecule is capable of delivering said antigen moiety to the antigen presenting cells of a host and capable of eliciting an immune response to

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said antigen moiety in the host, or a nucleic acid molecule comprising a first nucleotide sequence encoding a chain of a monoclonal antibody specific for a surface structure of antigen-presenting cells selected from the group consisting of the heavy chain and the light chain of the monoclonal antibody, a second nucleotide sequence encoding at least one antigen and a third nucleotide sequence comprising a promoter for eukaryotic cell expression of a fusion protein comprising said monoclonal antibody chain and said at least one antigen, wherein said at least one antigen is the selected antigen to at least one mouse to produce at least one immunized mouse;

(b) removing B-lymphocytes from the at least one immunized mouse;

(c) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(d) cloning the hybridomas;

(e) selecting clones which produce anti-selected antigen antibody;

(f) culturing the anti-selected antigen antibody-producing clones; and then

(g) isolating anti-selected antigen antibodies from the cultures.

ABSTRACT OF THE DISCLOSURE

- Antibody molecules specific for surface structures of antigen presenting cells that have been modified to include an antigen moiety at a specific site therein to produce novel conjugate antibody molecules are disclosed. These conjugate molecules are produced by genetic modification of genes encoding light and heavy chains of the surface structure specific antibody, and expression in mammalian cells to produce the conjugate antibody. The conjugate antibody retained specificity for antigen presenting cells and contained the antigen moiety. The conjugate antibody molecules deliver the antigen to antigen presenting cells to produce an enhanced immune response to a host immunized therewith.
- The conjugate antibody molecules and nucleic acid molecules encoding them are useful as antigens and as immunogens in diagnostic and prophylactic applications.

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Combined Declaration and Power of Attorney for United States Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, S.1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, S.119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed  
Yes No

(Number) (Country) (Day/Month/Year Filed)

I hereby claim the benefit under Title 35, United States Code, S.120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, S.112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, S.1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Appln. Serial No.) (Filing Date) (Status)  
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Peter W. McBurney, Reg. No. 19,352; Michael I. Stewart, Reg. No. 24,973; Thomas T. Rieder, Reg. No. 22,862; Roger T. Hughes, Reg. No. 25,265; John H. Woodley, Reg. No. 27,093; Stephen J. Perry, Reg. No. 32,107; Patricia A. Rae, Reg. No. 33,570, John R. Orange, Reg. No. 29,725 and David A. Ruston, Reg. No. 34,495.

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Send correspondence to:

SIM & McBURNEY  
Suite 701  
330 University Avenue  
Toronto, Ontario M5G 1R7, Canada

Direct telephone calls to:

Name: M.I. Stewart

at  
SIM & McBURNEY  
(416) 595-1155

Full name of sole or first inventor: Naveen N. Anand

Inventor's signature

Naveen N. Anand

June 22/95.

Date

Residence: Downsview, Ontario, Canada

Citizenship: India

Post Office Address: Apartment 117,  
1875 Steeles Avenue West,  
Downsview, Ontario,  
Canada, M3H 5T7

Full name of second inventor: Brian H. Barber

Inventor's signature

Brian H. Barber

June 22/95

Date

Residence: Mississauga, Ontario, Canada

Citizenship: Canadian

Post Office Address: 1428 Broadmoor Avenue,  
Mississauga, Ontario,  
Canada, L5G 3T5

Full name of third inventor: George <sup>A</sup>/<sub>E</sub> Cates

Inventor's signature

George Cates

June 22, 1995

Date

Residence: Richmond Hill, Ontario, Canada

Citizenship: Canadian

Post Office Address: 37 Pemberton Road,  
Richmond Hill, Ontario,  
Canada, L4C 3T5

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Full name of fourth inventor: Judith E. Caterini

Inventor's signature Judy Caterini

July 31, 1995  
Date

Residence: Ajax, Ontario, Canada

Citizenship: Canadian

Post Office Address: 91 Chatfield Drive,  
Ajax, Ontario,  
Canada, L1P 2J4

Full name of fifth inventor: Michel H. Klein

Inventor's signature M Klein

July 31, 1995  
Date

Residence: Willowdale, Ontario, Canada

Citizenship: Canadian

Post Office Address: 16 Munro Boulevard,  
Willowdale, Ontario,  
M2P 1B9, Canada

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Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
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(2) INFORMATION FOR SEQ ID NO:2:

SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

[illegible]

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 420 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTC CAAGCTGTGG TGTCTGTCC 60  
CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC 120  
ACTTGCACTG TCTCTGGGTT TTCATTAACC AGCTATGGTG TAACTGGGT TCGCCAGCCT 180  
CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT 240  
TCGGCTCTCA TGTCCAGACT GAGCATCAGC AAAGACAACT TCAAGAGCCA AGTTTTCTTA 300  
AAAATGAGCA GTCTGCAAAC TGATGACACA GCCATGTACT ACTGTGCCAG AGCCTATGGT 360  
GACTACGTCC ACTATGCTAT GGAATACTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA 420

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 140 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys  
1 5 10 15  
Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val  
20 25 30  
Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser  
35 40 45  
Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly  
50 55 60  
Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn  
65 70 75 80  
Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser  
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met  
 100 105 110

Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp  
 115 120 125

Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser  
 130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn  
 1 5 10 15

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr  
 20 25 30

Lys Asn

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 108 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCCTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60

ATACATATAG GGCCTGGTAG GGCTTTTTAT ACTACTAAGA ATTAATAA 108

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 60 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT

60

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCCTACCAG GCCCTATATG TATCCTCTTC CTCTTATTCT TATAAAACCT A

51

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGGCCTGGT AGGGCTTTTT ATACTACTAA GAATTAATAA AAGCTTTAGC G

51

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATTATGGAT CCGGTCCTAA

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTCAGGTACC GGTCTTAAAG AACCTTTTAG

30

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCTAAAGCT TTTATTAATT C

21

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCCTAAGCT TCCGCCATGG ACATGAGGGT TCCTGCTC

38

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCGTTTCAGC TCGAGCTTGG TCCCAGCACC GAA

33

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTACTCGAG CTGAAACGGA CTGTGGCTGC ACCATCTGTC

40

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 44 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATTAAAGCTT TTACTAGGAT CCACACTCTC CCCTGTTGAA GCTC

44

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCTAAGCTT CCGCCATGGC TCTCCTGGTA CTGTTC

36

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCGCACTAGT TCCTTGACCC CAGTAGTCC

29

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 52 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCGCACTAGT GTCACCGCCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT TC

52

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 43 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ACGCAAGCTT TTACTAGGTA CCTTTACCCG GAGACAGGGA GAG

43

Seq ID: 182060

~~A~~ V<sub>L</sub> sequence of the light chain of murine 44H104 mab:

ATG GAC ATG AGG GTT CCT GCT CAC GTT TTT GGC TTC TTG TTG CTC TGG TTT  
MET Asp MET Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp Phe  
CCA GGT ACC AGA TGT GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC TTA TCT  
Pro Gly Thr Arg Cys Asp Ile Gln MET Thr Gln Ser Pro Ser Ser Leu Ser  
GCC TCT CTG GGA CAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG GAA ATT  
Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu Ile  
AGT GGT TAC TTA ACC TGG CTT CAG CAG AAA CCA GAT GGA ACT ATT AAA CGC  
Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg  
CTG GTC TAC GCC GCG TCC ACT TTA GAT TCT GGT GTC CCA AAA AGG TTC AGT  
Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val Pro Lys Arg Phe Ser  
GGC AGT AGG TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTT GAG TCT  
Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser  
GAA GAT TTT GCA GAC TAT TAC TGT CTA CAA TAT ACT AAT TAT CCG CTC ACG  
Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln Tyr Thr Asn Tyr Pro Leu Thr  
TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA  
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys

FIG. 1A

~~B~~ V<sub>H</sub> sequence of the heavy chain of murine 44H104 mab:

ATG GCT CTC CTG GTA CTG TTC CTC TCC CTG GCT GCA TTT CCA AGC TGT GGT  
MET Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys Gly  
GTC CTG TCC CAG GTG CAG CTG AAG GAG TCA GGA CCT GGC CTG GTG GCG CCC  
Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro  
TCA CAG AGC CTG TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA TTA ACC AGC  
Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser  
TAT GGT GTA CAC TGG GTT CGC CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG  
Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
GGA GTA ATA TGG GCT GGT GGA AGC ATA AAT TAT AAT TCG GCT CTC ATG TCC  
Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn Ser Ala Leu MET Ser  
AGA CTG AGC ATC AGC AAA GAC AAC TTC AAG AGC CAA GTT TTC TTA AAA ATG  
Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser Gln Val Phe Leu Lys MET  
AGC AGT CTG CAA ACT GAT GAC ACA GCC ATG TAC TAC TGT GCC AGA GCC TAT  
Ser Ser Leu Gln Thr Asp Asp Thr Ala MET Tyr Tyr Cys Ala Arg Ala Tyr  
GGT GAC TAC GTC CAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC  
Gly Asp Tyr Val His Tyr Ala MET Asp Tyr Trp Gly Gln Gly Thr Ser Val  
ACC GCC TCC TCA  
Thr Ala Ser Ser

FIG. 1B

Figure 1

Figure 1

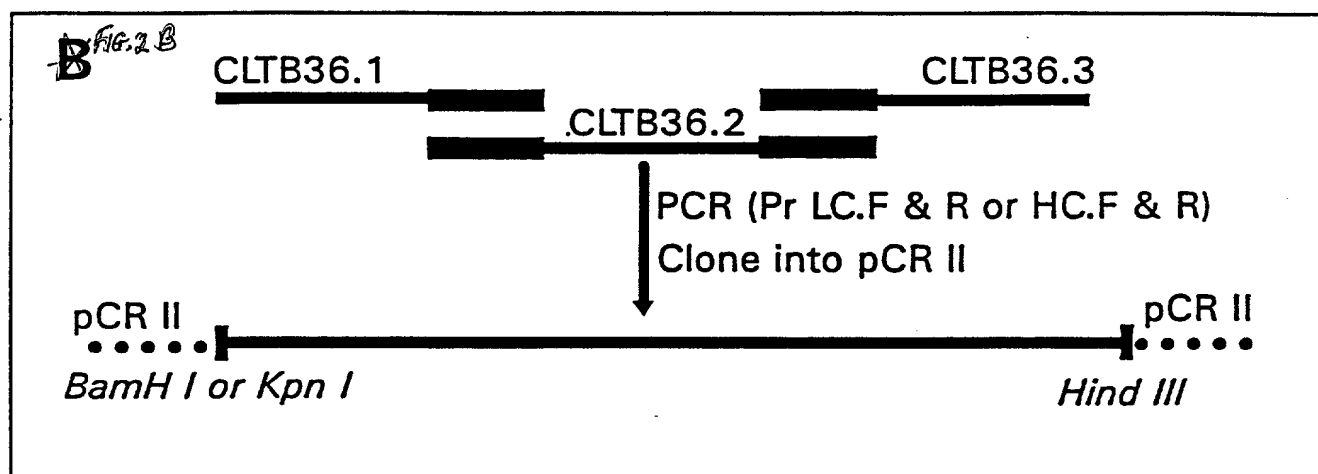
09007093-01498

# Assembly of gene encoding CLTB36

**A** Fig. 2A

Amino acid seq. GPKEPFRDYVDRFYK NKRKRIHIGPGRAFYTTKN

Gene seq. GGTCCTAAAGAACCTTTTAGAGACTATGTTGATAGGTTTTA  
TAAGAATAAGAGGAAGAGGATACATATAGGGCCTGGT  
AGGGCTTTTTTATACTACTAAGAATTAATAA



**C** Fig. 2C

CLTB36.1 CATTATGGATCCGGTCCTAAAGAACCTTTTAGAGACTATGTTGAT  
AGGTTTTATAAGAAT

CLTB36.2 GCCCTACCAGGCCCTATATGTATCCTCTTCCTCTTATTCTTATAAA  
ACCTA

CLTB36.3 AGGGCCTGGTAGGGCTTTTTATACTACTAAGAATTAATAAAAGCT  
TTAGCG

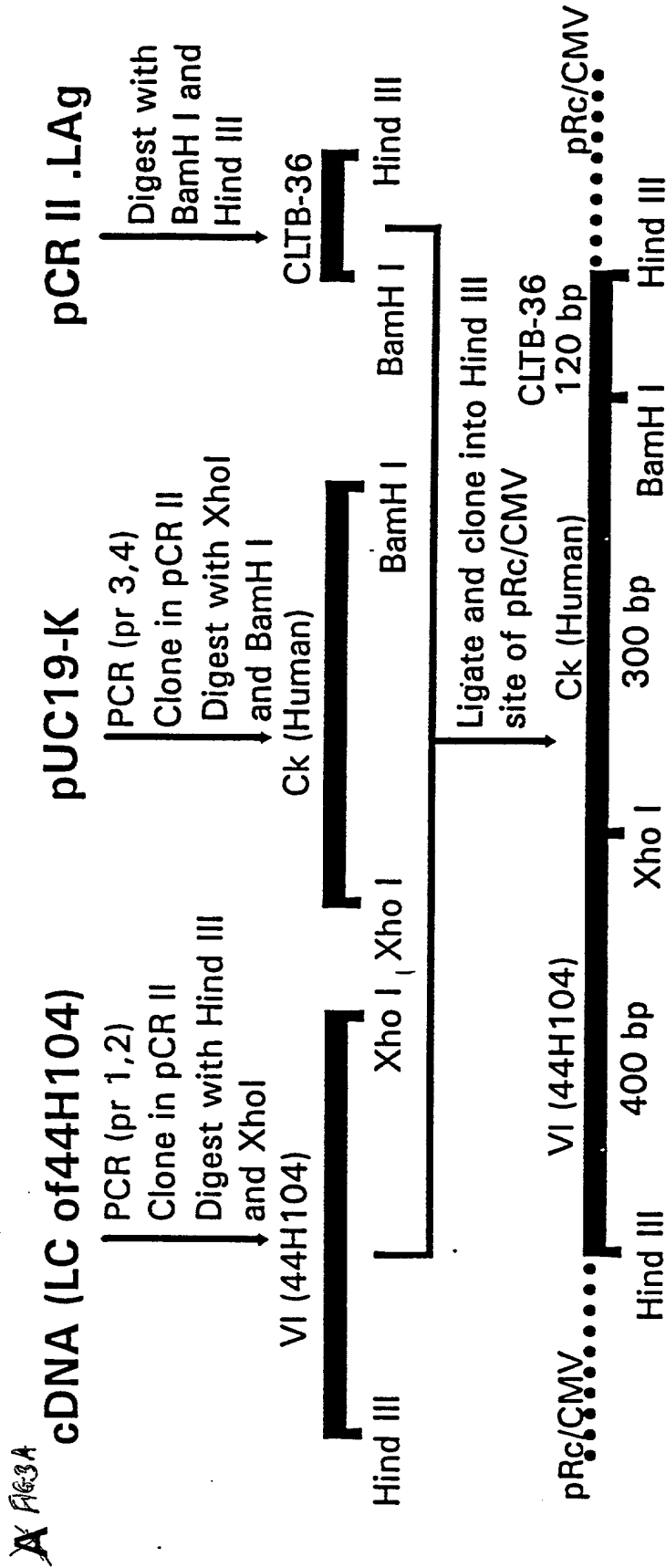
Pr LC.F *BamH I*  
CATTATGGATCCGGTCCTAA

Pr HC.F *Kpn I*  
GTCAGGTACCGGTCCTAAAGAACCTTTTAG

Pr R *Hind III*  
GGCTAAAGCTTTTATTAATTC

~~Figure 2~~

# Assembly of chimeric 44H104-CLTB36 light chain gene



## **B** **Fig 3B**

*Hind III*

Pr. 1 AGCCTAAGCTTCCGCCATGGACATGAGGGTTCCTGCTC

*Xho I*

Pr. 2 CCGTTTCAGCTCGAGCTTGGTCCAGACCGAA

*Xho I*

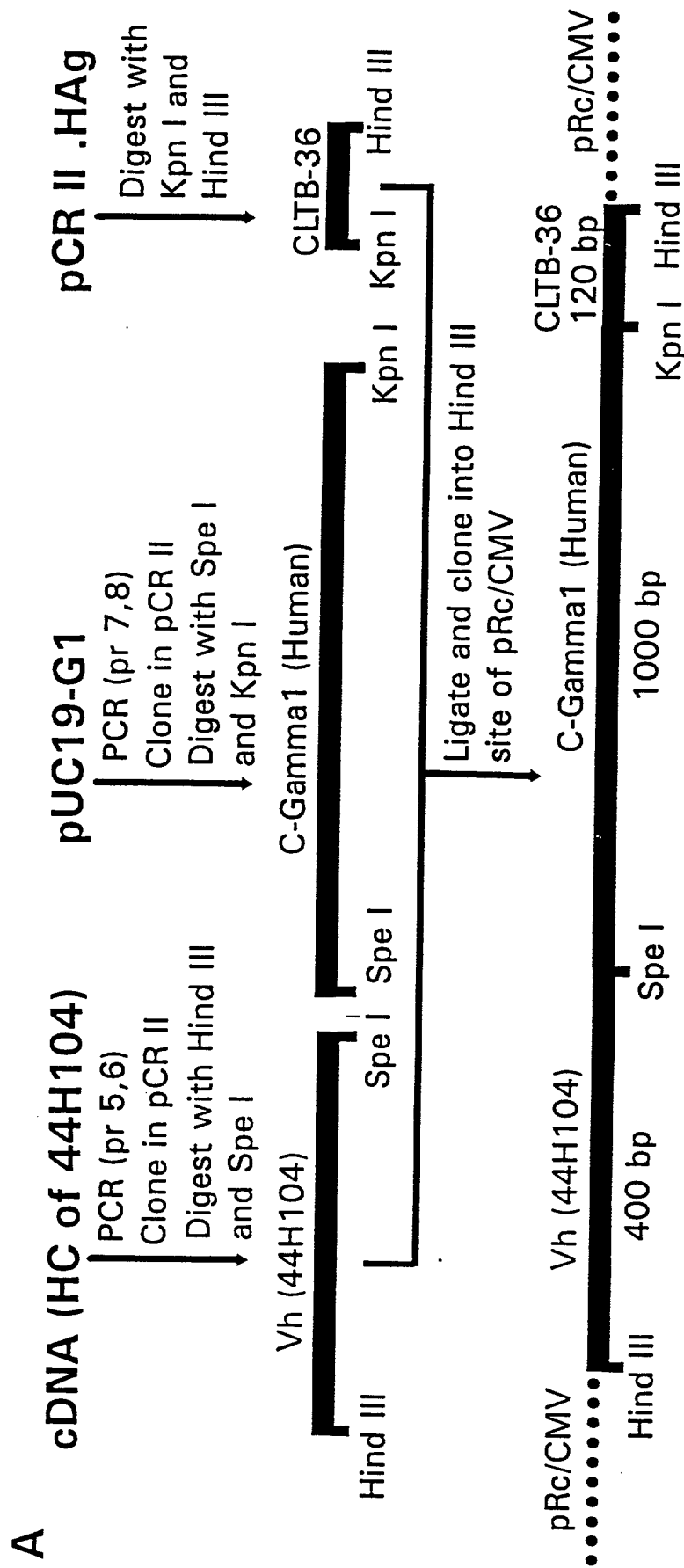
Pr. 3 CCTACTCGAGCTGAAACGGACTGTGGCTGCACCATCTGTC

*BamH I*

Pr. 4 ATTAAAGCTTTTACTAGGATCCACACTCTCCCTGTTGAAGCTC

**Figure 3.**

# Assembly of chimeric 44H104-CLTB36 heavy chain gene



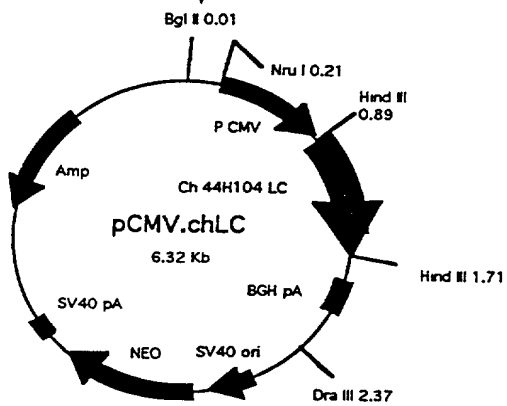
**B**

*Hind III*  
Pr. 5 AGCTAAGCIIICCGCCATGGCTCTCCTGGTACTGTTC  
*Spe I*  
Pr. 6 GCGCACTAGITCCTTGACCCAGTAGTCC  
*Spe I*  
Pr. 7 GCGCACTAGITGTCACCGCCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTC  
*Hind III*  
Pr. 8 ACGCAAGCIIITACTAGGTACCTTACCCGGAGACAGGGAGAG

Figure 4

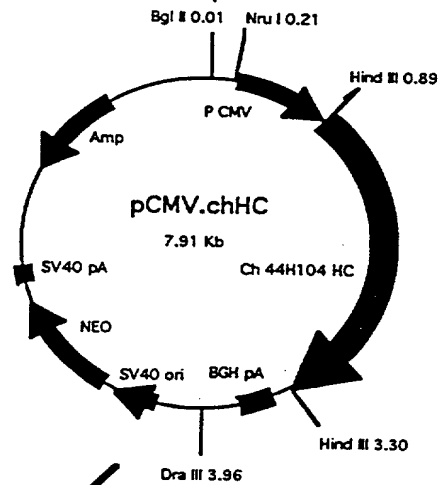
Hind III fragment (ch. LC)  
pRc/CMV cut with Hind III

Ligate



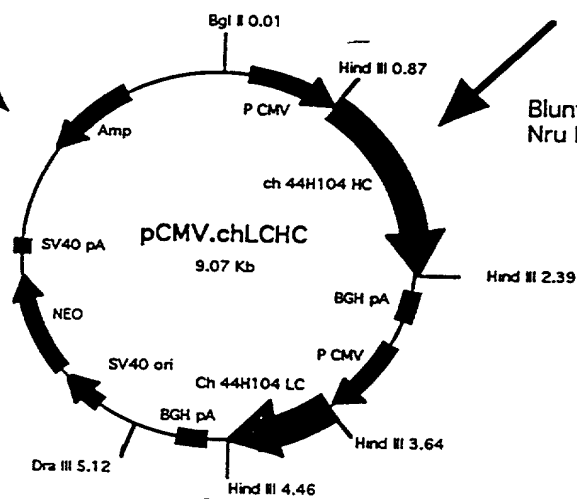
Hind III fragment (ch. HC)  
pRc/CMV cut with Hind III

Ligate



Digested  
with Nru I

Blunt ended 2.8 Kb  
Nru I/Dra III Fr



Digest with Bgl II

Blunt ended 1.9 Kb Pvu II/BamH I  
fragment from pSV2.dhfr

Ligate

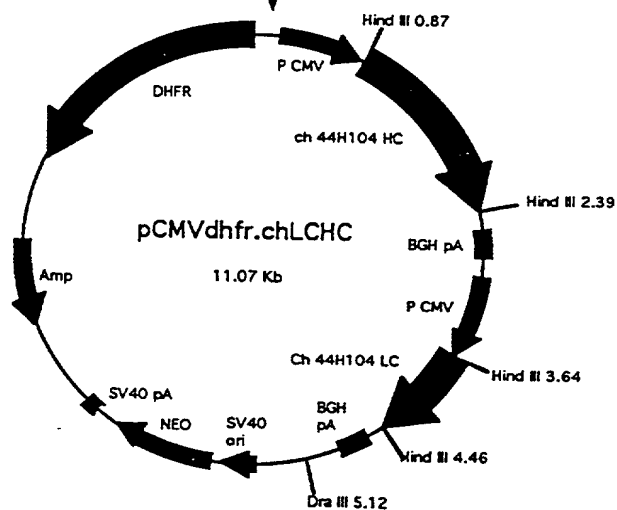


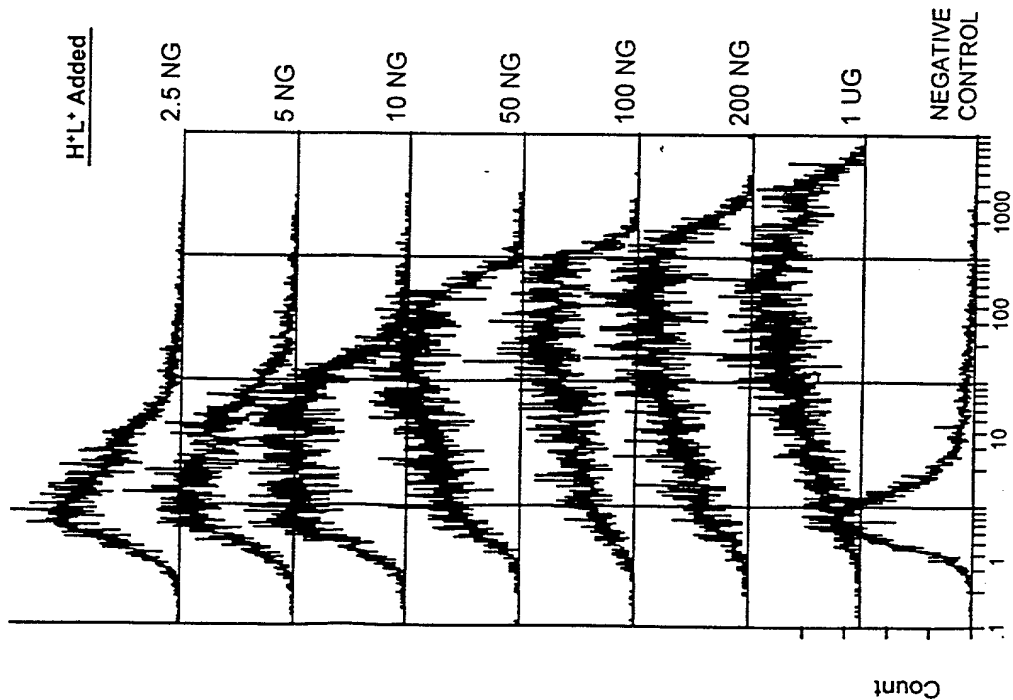
Figure 5

bioRxiv preprint doi: <https://doi.org/10.1101/000000>; this version posted October 1, 2014. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

# Epitope Recognition on Recombinant H<sup>+</sup>L<sup>+</sup> Human Chimeric Antibody

## Bound to HLA-DR

A. Anti-Human IgG



B. Anti-CLTB-36

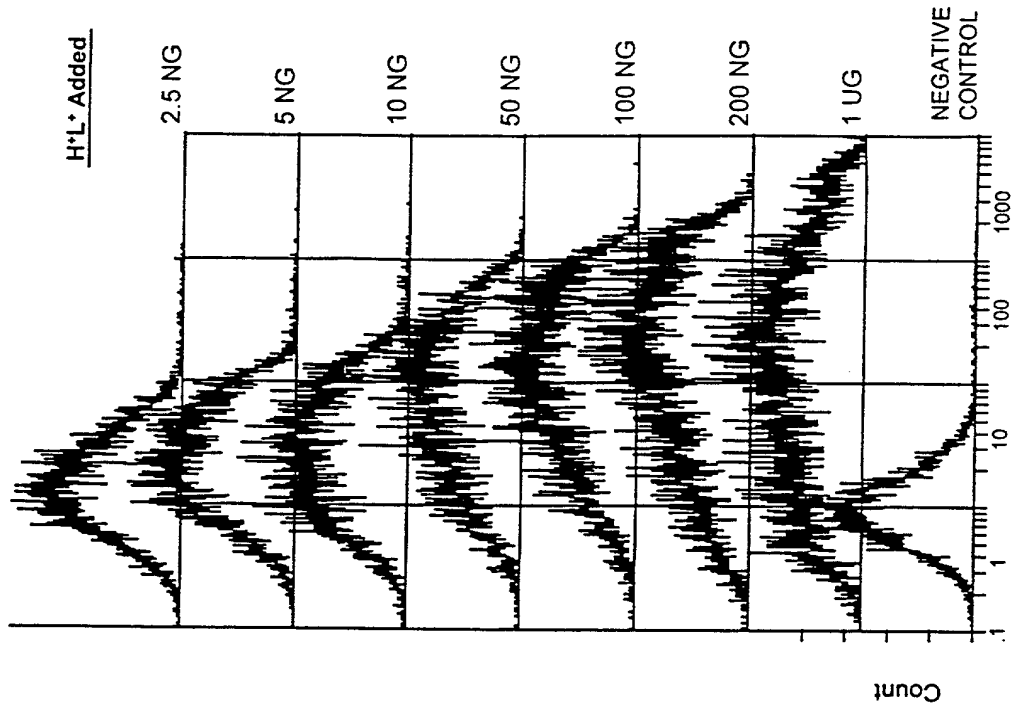
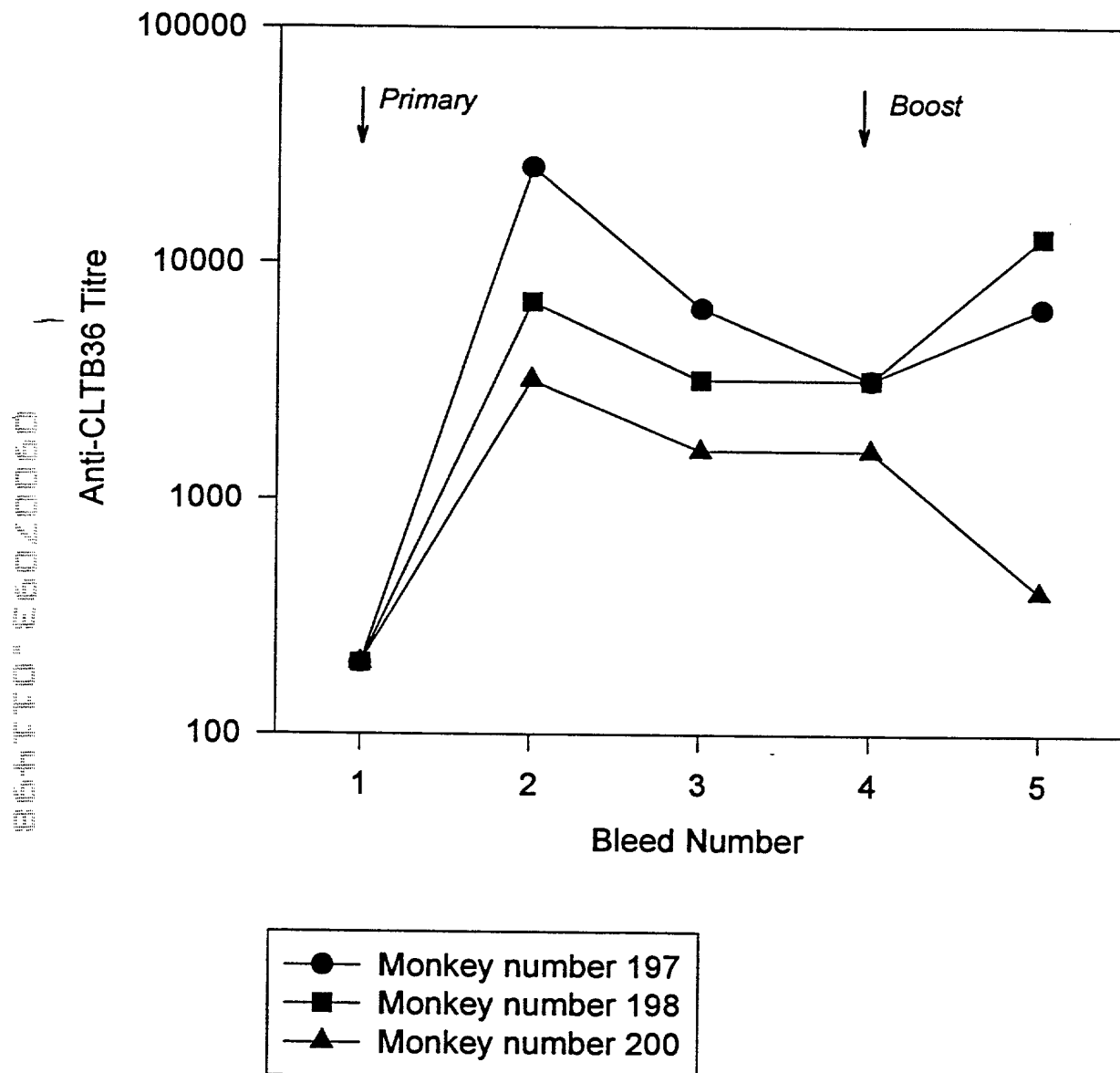


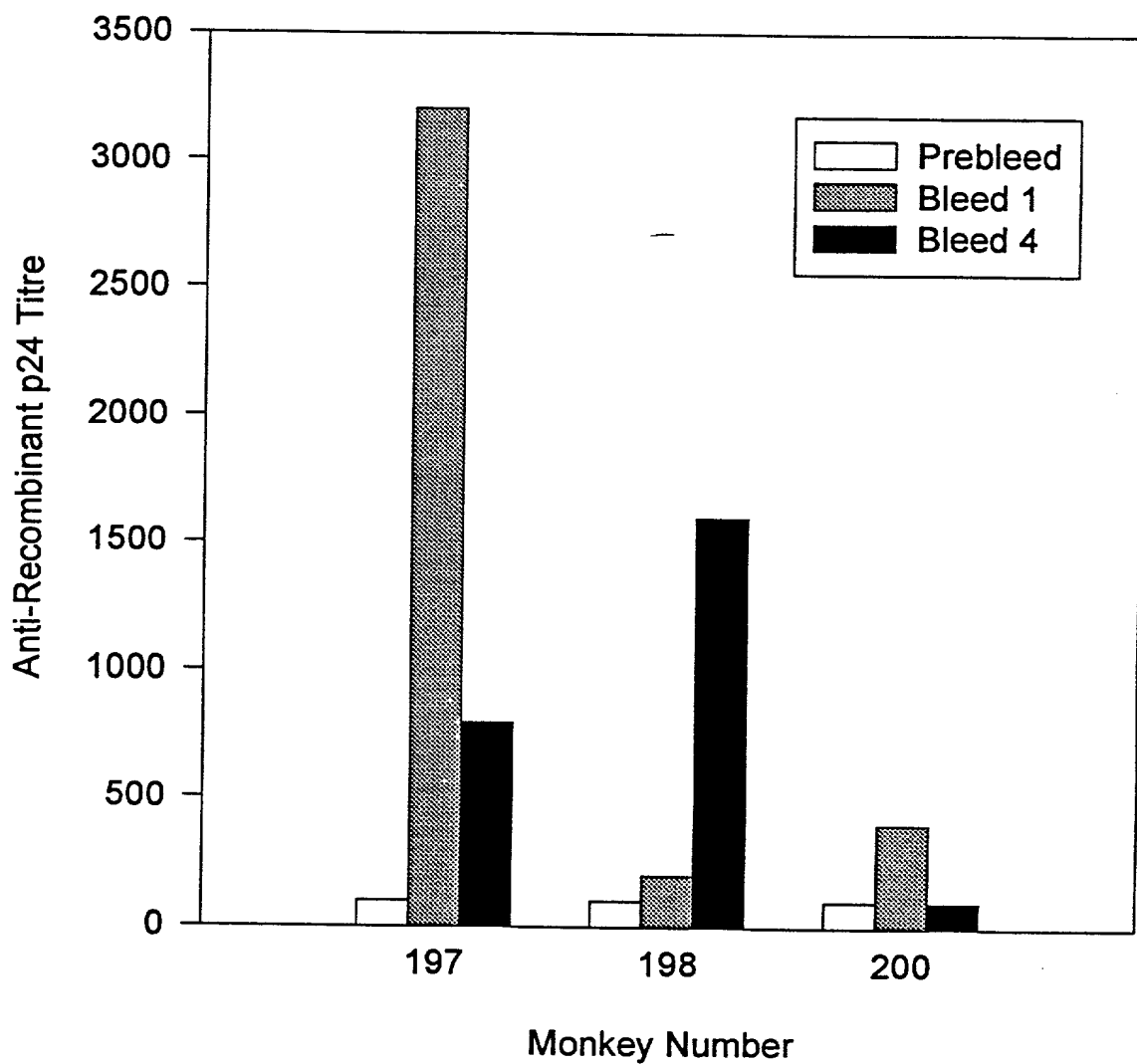
Figure 6



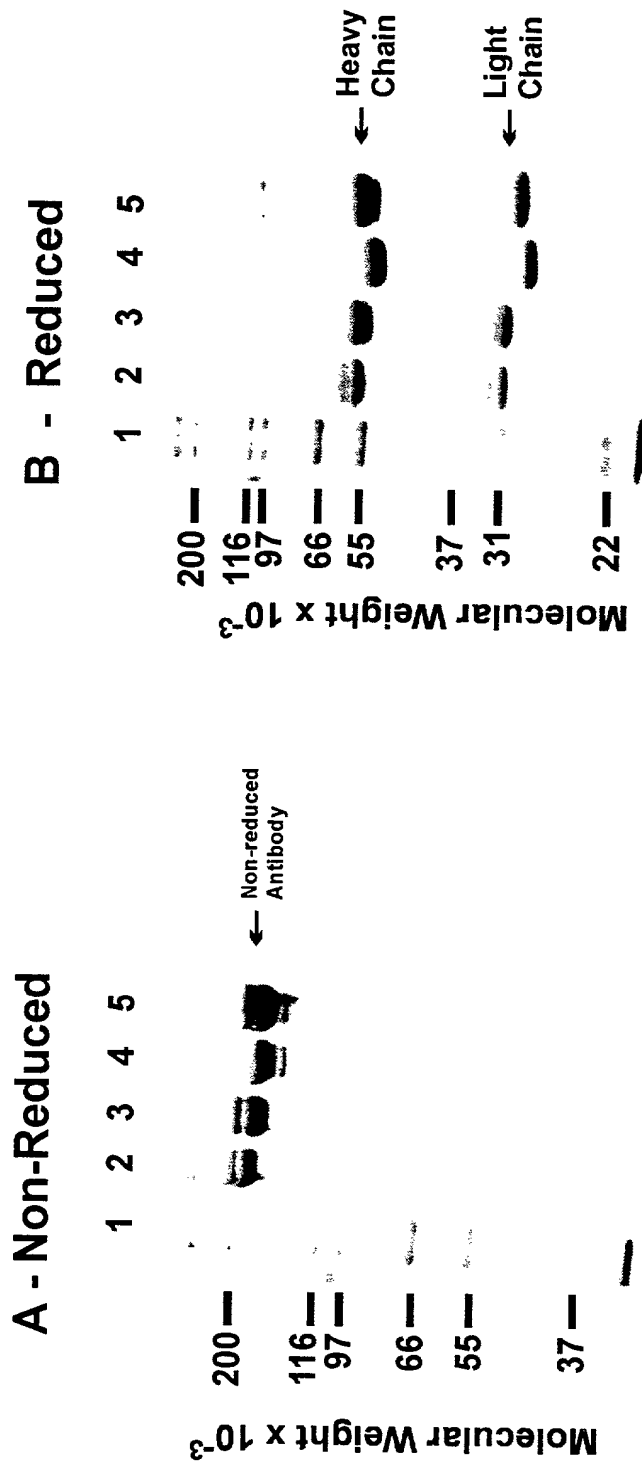
Figure 9 - Anti-CLTB36 Titres



~~108~~  
Figure ~~10~~ - Anti-Recombinant p24 Titres

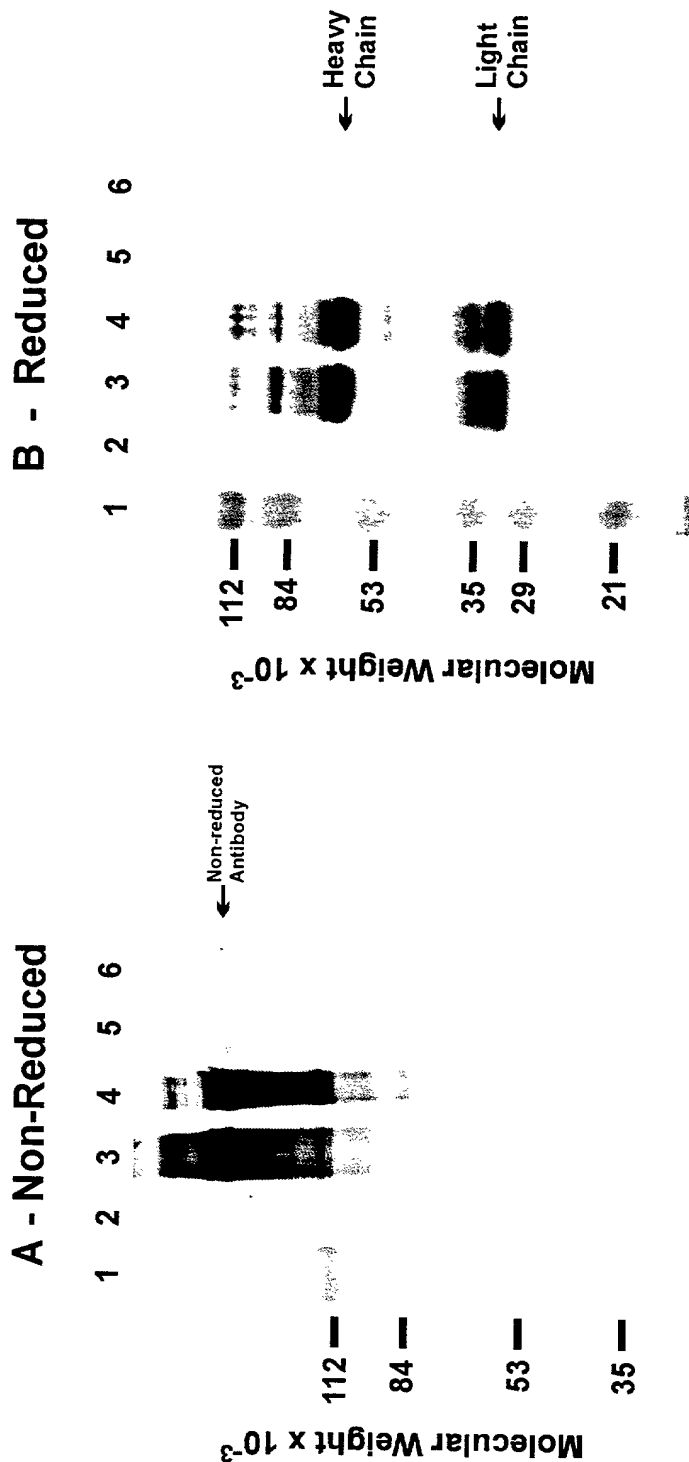


## Coomassie Blue stained Polyacrylamide Gels of Recombinant Targeting Antibody



Lane 1 Molecular weight standards  
Lane 2 Recombinant targeting antibody - Protein A purified  
Lane 3 Recombinant targeting antibody - gel filtration purified  
Lane 4 Mouse monoclonal antibody 44H104  
Lane 5 Human IgG<sub>1</sub>

# Western blots of recombinant targeting antibody probed with guinea pig anti-CLTB36 antiserum



Lane 1 Molecular weight standards  
Lane 2 Blank lane  
Lane 3 Recombinant targeting antibody - Protein A purified  
Lane 4 Recombinant targeting antibody - gel filtration purified  
Lane 5 Mouse monoclonal antibody 44H104  
Lane 6 Human IgG<sub>1</sub>

# CRF Errors Corrected by the STIC Systems Branch

Serial Number: 09/007,093

CRF Processing Date: 2/20/98  
 Edited by: AS  
 Verified by: AS (STIC staff)

- ☐ Changed a file from non-ASCII to ASCII
- ☐ Changed the margins in cases where the sequence text was "wrapped" down to the next line.
- ☐ Edited a format error in the Current Application Data section, specifically: \_\_\_\_\_
- ☐ Edited the Current Application Data section with the actual current number. The number inputted by the applicant was ☐ the prior application data; or ☐ other \_\_\_\_\_
- ☐ Added the mandatory heading and subheadings for "Current Application Data".
- ☐ Edited the "Number of Sequences" field. The applicant spelled out a number instead of using an integer.
- ☒ Changed the spelling of a mandatory field (the headings or subheadings), specifically:  
"APPLICATION" under (iii) PRIOR APP DATA
- ☐ Corrected the SEQ ID NO when obviously incorrect. The sequence numbers that were edited were: \_\_\_\_\_
- ☐ Inserted or corrected a nucleic number at the end of a nucleic line. SEQ ID NO's edited: \_\_\_\_\_
- ☐ Corrected subheading placement. All responses must be on the same line as each subheading. If the applicant placed a response below the subheading, this was moved to its appropriate place.
- ☐ Inserted colons after headings/subheadings. Headings edited included: \_\_\_\_\_
- ☐ Deleted extra, invalid, headings used by an applicant, specifically: \_\_\_\_\_
- ☐ Deleted: ☐ non-ASCII "garbage" at the beginning/end of files; ☐ secretary initials/filename at end of file;  
☐ page numbers throughout text; ☐ other invalid text, such as \_\_\_\_\_
- ☐ Inserted mandatory headings, specifically: \_\_\_\_\_
- ☐ Corrected an obvious error in the response, specifically: \_\_\_\_\_
- ☐ Edited identifiers where upper case is used but lower case is required, or vice versa.
- ☐ Corrected an error in the Number of Sequences field, specifically: \_\_\_\_\_
- ☐ A "Hard Page Break" code was inserted by the applicant. All occurrences had to be deleted.
- ☐ Deleted **ending** stop codon in amino acid sequences and adjusted the "(A)Length:" field accordingly (error due to a PatentIn bug). Sequences corrected: \_\_\_\_\_
- ☐ Other: \_\_\_\_\_

\*Examiner: The above corrections must be communicated to the applicant in the first Office Action. DO NOT send a copy of this form.

SEQUENCE LISTING

(1) General Information:

(i) APPLICANT: Anand, Naveen N  
Barber, Brian H  
Cates, George A  
Caterini, Judith E  
Klein, Michel H

(ii) TITLE OF INVENTION: CHIMERIC ANTIBODIES FOR DELIVERY OF  
ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Sim & McBurney  
(B) STREET: Suite 701, 330 University Avenue  
(C) CITY: Toronto  
(D) STATE: Ontario  
(E) COUNTRY: Canada  
(F) ZIP: M5G 1R7

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/483,576  
(B) FILING DATE: 07-JUN-1995

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Stewart, Michael I  
(B) REGISTRATION NUMBER: 24,973  
(C) REFERENCE/DOCKET NUMBER: 1038-765

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (416) 595-1155  
(B) TELEFAX: (416) 595-1163

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48

49 (2) INFORMATION FOR SEQ ID NO:1:

50

51 (i) SEQUENCE CHARACTERISTICS:

52 (A) LENGTH: 387 base pairs

53 (B) TYPE: nucleic acid

54 (C) STRANDEDNESS: single

55 (D) TOPOLOGY: linear

56

57

58

59

60

61 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

62

63 ATGGACATGA GGGTTCCTGC TCACGTTTTT GGCTTCTTGT TGCTCTGGTT TCCAGGTACC 60

64

65 AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCCTTAT CTGCCTCTCT GGGACAAAGA 120

66

67 GTCAGTCTCA CTTGTCGGGC AAGTCAGGAA ATTAGTGGTT ACTTAACCTG GCTTCAGCAG 180

68

69 AAACCAGATG GAACTATTAA ACGCCTGGTC TACGCCGCGT CCACTTTAGA TTCTGGTGTC 240

70

71 CCAAAAAGGT TCAGTGGCAG TAGGTCTGGG TCAGATTATT CTCTCACCAT CAGCAGCCTT 300

72

73 GAGTCTGAAG ATTTTGCAGA CTATTACTGT CTACAATATA CTAATTATCC GCTCACGTTT 360

74

75 GGTGCTGGGA CCAAGCTGGA GCTGAAA 387

76

77 (2) INFORMATION FOR SEQ ID NO:2:

78

79 (i) SEQUENCE CHARACTERISTICS:

80 (A) LENGTH: 129 amino acids

81 (B) TYPE: amino acid

82 (C) STRANDEDNESS: single

83 (D) TOPOLOGY: linear

84

85

86

87

88

89 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

90

91 Met Asp Met Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp

92 1 5 10 15

93

94 Phe Pro Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser

95 20 25 30

96

97 Leu Ser Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser

98 35 40 45

99

B67F01/000000

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100 Gln Glu Ile Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly  
101 50 55 60  
102  
103 Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val  
104 65 70 75 80  
105  
106 Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Thr Ser Leu Thr  
107 85 90 95  
108  
109 Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln  
110 100 105 110  
111  
112 Tyr Thr Asn Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu  
113 115 120 125  
114  
115 Lys  
116  
117  
118

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 420 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

131  
132  
133 ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTT CAAGCTGTGG TGTCTGTGCC 60  
134  
135 CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC 120  
136  
137 ACTTGCACTG TCTCTGGGTT TTCATTAACC AGCTATGGTG TACTGTGGGT TCGCCAGCCT 180  
138  
139 CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT 240  
140  
141 TCGGCTCTCA TGTCCAGACT GAGCATCAGC AAAGACAACT TCAAGAGCCA AGTTTTCTTA 300  
142  
143 AAAATGAGCA GTCTGCAAAC TGATGACACA GCCATGTACT ACTGTGCCAG AGCCTATGGT 360  
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145 GACTACGTCC ACTATGCTAT GGACTIONTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA 420  
146  
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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids  
(B) TYPE: amino acid



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PATENT APPLICATION *US/09/007,093*

DATE: 02/24/98  
TIME: 11:44:46

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153 (C) STRANDEDNESS: single
154 (D) TOPOLOGY: linear
155
156
157
158
159
160 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
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162 Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys
163 1 5 10 15
164
165 Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val
166 20 25 30
167
168 Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
169 35 40 45
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171 Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly
172 50 55 60
173
174 Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn
175 65 70 75 80
176
177 Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser
178 85 90 95
179
180 Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met
181 100 105 110
182
183 Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp
184 115 120 125
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186 Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser
187 130 135 140
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189 (2) INFORMATION FOR SEQ ID NO:5:
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191 (i) SEQUENCE CHARACTERISTICS:
192 (A) LENGTH: 34 amino acids
193 (B) TYPE: amino acid
194 (C) STRANDEDNESS: single
195 (D) TOPOLOGY: linear
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197
198
199
200
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203 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn
204 1 5 10 15
205

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PATENT APPLICATION US/09/007,093DATE: 02/24/98  
TIME: 11:44:49

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206 Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr  
207 20 25 30  
208

209 Lys Asn  
210  
211

212 (2) INFORMATION FOR SEQ ID NO:6:  
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214 (i) SEQUENCE CHARACTERISTICS:

215 (A) LENGTH: 108 base pairs

216 (B) TYPE: nucleic acid

217 (C) STRANDEDNESS: single

218 (D) TOPOLOGY: linear  
219  
220  
221  
222  
223

224 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

225  
226 GGTCCCTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60

227  
228 ATACATATAG GGCCTGGTAG GGCTTTTTAT ACTACTAAGA ATTAATAA 108  
229

230 (2) INFORMATION FOR SEQ ID NO:7:  
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232 (i) SEQUENCE CHARACTERISTICS:

233 (A) LENGTH: 60 base pairs

234 (B) TYPE: nucleic acid

235 (C) STRANDEDNESS: single

236 (D) TOPOLOGY: linear  
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238  
239  
240  
241

242 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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244 CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT 60  
245  
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247 (2) INFORMATION FOR SEQ ID NO:8:  
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249 (i) SEQUENCE CHARACTERISTICS:

250 (A) LENGTH: 51 base pairs

251 (B) TYPE: nucleic acid

252 (C) STRANDEDNESS: single

253 (D) TOPOLOGY: linear  
254  
255  
256  
257  
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B64T0-602060

[illegible]

**SEQUENCE VERIFICATION REPORT**  
**PATENT APPLICATION** *US/09/007,093*

DATE: 02/24/98  
TIME: 11:44:53

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Original Text

**RAW SEQUENCE LISTING**  
**PATENT APPLICATION US/09/007,093**

DATE: 02/20/98  
TIME: 12:15:37

**INPUT SET: S23619.raw**

**This Raw Listing contains the General Information Section and up to the first 5 pages.**

## SEQUENCE LISTING

(1) General Information:

(i) **APPLICANT:** Anand, Naveen N  
Barber, Brian H  
Cates, George A  
Caterini, Judith E  
Klein, Michel H

Does Not Comply  
Corrected Diskette Needed

(ii) TITLE OF INVENTION: CHIMERIC ANTIBODIES FOR DELIVERY OF ANTIGENS TO SELECTED CELLS OF THE IMMUNE SYSTEM

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Sim & McBurney  
(B) STREET: Suite 701, 330 University Avenue  
(C) CITY: Toronto  
(D) STATE: Ontario  
(E) COUNTRY: Canada  
(F) ZIP: M5G 1R7

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/483,576  
(B) FILING DATE: 07-JUN-1995

~~(C) CLASSIFICATION:~~

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Stewart, Michael I  
(B) REGISTRATION NUMBER: 24,973  
(C) REFERENCE/DOCKET NUMBER: 1038-765

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (416) 595-1155

RAW SEQUENCE LISTING  
PATENT APPLICATION US/09/007,093DATE: 02/20/98  
TIME: 12:15:40

INPUT SET: S23619.raw

47 (B) TELEFAX: (416) 595-1163

48

49

50 (2) INFORMATION FOR SEQ ID NO:1:

51

52 (i) SEQUENCE CHARACTERISTICS:

53 (A) LENGTH: 387 base pairs

54 (B) TYPE: nucleic acid

55 (C) STRANDEDNESS: single

56 (D) TOPOLOGY: linear

57

58

59

60

61

62 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

63

64 ATGGACATGA GGGTTCCTGC TCACGTTTTT GGCTTCTTGT TGCTCTGGTT TCCAGGTACC 60

65

66 AGATGTGACA TCCAGATGAC CCAGTCTCCA TCCTCCTTAT CTGCCTCTCT GGGACAAAGA 120

67

68 GTCAGTCTCA CTTGTCGGGC AAGTCAGGAA ATTAGTGGTT ACTTAACCTG GCTTCAGCAG 180

69

70 AAACCAGATG GAACTATTAA ACGCCTGGTC TACGCCGCGT CCACTTTAGA TTCTGGTGTC 240

71

72 CCAAAAAGGT TCACTGGCAG TAGGTCTGGG TCAGATTATT CTCTCACCAT CAGCAGCCTT 300

73

74 GAGTCTGAAG ATTTTGCAGA CTATTACTGT CTACAATATA CTAATTATCC GCTCACGTTC 360

75

76 GGTGCTGGGA CCAAGCTGGA GCTGAAA 387

77

78 (2) INFORMATION FOR SEQ ID NO:2:

79

80 (i) SEQUENCE CHARACTERISTICS:

81 (A) LENGTH: 129 amino acids

82 (B) TYPE: amino acid

83 (C) STRANDEDNESS: single

84 (D) TOPOLOGY: linear

85

86

87

88

89

90 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

91

92 Met Asp Met Arg Val Pro Ala His Val Phe Gly Phe Leu Leu Leu Trp

93 1 5 10 15

94

95 Phe Pro Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser

96 20 25 30

97

98 Leu Ser Ala Ser Leu Gly Gln Arg Val Ser Leu Thr Cys Arg Ala Ser

99 35 40 45

RAW SEQUENCE LISTING  
PATENT APPLICATION US/09/007,093DATE: 02/20/98  
TIME: 12:15:44

INPUT SET: S23619.raw

100  
101 Gln Glu Ile Ser Gly Tyr Leu Thr Trp Leu Gln Gln Lys Pro Asp Gly  
102 50 55 60  
103  
104 Thr Ile Lys Arg Leu Val Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val  
105 65 70 75 80  
106  
107 Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Thr Ser Leu Thr  
108 85 90 95  
109  
110 Ile Ser Ser Leu Glu Ser Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln  
111 100 105 110  
112  
113 Tyr Thr Asn Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu  
114 115 120 125  
115  
116 Lys  
117  
118  
119

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 420 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

132  
133  
134 ATGGCTCTCC TGGTACTGTT CCTCTCCCTG GCTGCATTTT CAAGCTGTGG TGTCCTGTCC 60  
135  
136 CAGGTGCAGC TGAAGGAGTC AGGACCTGGC CTGGTGGCGC CCTCACAGAG CCTGTCCATC 120  
137  
138 ACTTGCACTG TCTCTGGGTT TTCATTAACC AGCTATGGTG TACTGTGGGT TCGCCAGCCT 180  
139  
140 CCAGGAAAGG GTCTGGAGTG GCTGGGAGTA ATATGGGCTG GTGGAAGCAT AAATTATAAT 240  
141  
142 TCGGCTCTCA TGTCCAGACT GAGCATCAGC AAAGACAACT TCAAGAGCCA AGTTTTCTTA 300  
143  
144 AAAATGAGCA GTCTGCAAAC TGATGACACA GCCATGTACT ACTGTGCCAG AGCCTATGGT 360  
145  
146 GACTACGTCC ACTATGCTAT GGACTACTGG GGTCAAGGAA CCTCAGTCAC CGCCTCCTCA 420  
147  
148

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids

**RAW SEQUENCE LISTING**  
PATENT APPLICATION *US/09/007,093*

DATE: 02/20/98

TIME: 12:15:47

**INPUT SET: S23619.raw**

```

153      (B) TYPE: amino acid
154      (C) STRANDEDNESS: single
155      (D) TOPOLOGY: linear
156
157
158
159
160
161      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
162
163      Met Ala Leu Leu Val Leu Phe Leu Ser Leu Ala Ala Phe Pro Ser Cys
164      1              5              10              15
165
166      Gly Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val
167              20              25              30
168
169      Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
170              35              40              45
171
172      Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly
173      50              55              60
174
175      Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Ile Asn Tyr Asn
176      65              70              75              80
177
178      Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Phe Lys Ser
179              85              90              95
180
181      Gln Val Phe Leu Lys Met Ser Ser Leu Gln Thr Asp Asp Thr Ala Met
182              100              105              110
183
184      Tyr Tyr Cys Ala Arg Ala Tyr Gly Asp Tyr Val His Tyr Ala Met Asp
185              115              120              125
186
187      Tyr Trp Gly Gln Gly Thr Ser Val Thr Ala Ser Ser
188      130              135              140
189
190      (2) INFORMATION FOR SEQ ID NO:5:
191
192      (i) SEQUENCE CHARACTERISTICS:
193      (A) LENGTH: 34 amino acids
194      (B) TYPE: amino acid
195      (C) STRANDEDNESS: single
196      (D) TOPOLOGY: linear
197
198
199
200
201
202      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
203
204      Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Asn
205      1              5              10              15

```

RAW SEQUENCE LISTING  
PATENT APPLICATION US/09/007,093DATE: 02/20/98  
TIME: 12:15:51

INPUT SET: S23619.raw

206  
207 Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr  
208 20 25 30  
209  
210 Lys Asn  
211  
212

## 213 (2) INFORMATION FOR SEQ ID NO:6:

214  
215 (i) SEQUENCE CHARACTERISTICS:  
216 (A) LENGTH: 108 base pairs  
217 (B) TYPE: nucleic acid  
218 (C) STRANDEDNESS: single  
219 (D) TOPOLOGY: linear  
220  
221  
222  
223  
224

## 225 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

226  
227 GGTCTTAAAG AACCTTTTAG AGACTATGTT GATAGGTTTT ATAAGAATAA GAGGAAGAGG 60  
228  
229 ATACATATAG GGCCTGGTAG GGCTTTTTTAT ACTACTAAGA ATTAATAA 108  
230

## 231 (2) INFORMATION FOR SEQ ID NO:7:

232  
233 (i) SEQUENCE CHARACTERISTICS:  
234 (A) LENGTH: 60 base pairs  
235 (B) TYPE: nucleic acid  
236 (C) STRANDEDNESS: single  
237 (D) TOPOLOGY: linear  
238  
239  
240  
241  
242

## 243 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

244  
245 CATTATGGAT CCGGTCCTAA AGAACCTTTT AGAGACTATG TTGATAGGTT TTATAAGAAT 60  
246  
247

## 248 (2) INFORMATION FOR SEQ ID NO:8:

249  
250 (i) SEQUENCE CHARACTERISTICS:  
251 (A) LENGTH: 51 base pairs  
252 (B) TYPE: nucleic acid  
253 (C) STRANDEDNESS: single  
254 (D) TOPOLOGY: linear  
255  
256  
257  
258



Line	Error	Original Text
35	Unknown or Misplaced Identifier	(vii) PRIOR APPLICATION DATA:
36	Wrong application Serial Number	(A) APPLICATION NUMBER: US 08/483,576

Category	Item	Value
General Information	Age	25.00
	Gender	Male
	Height	1.75
	Weight	70.00
	Education	High School
	Occupation	Student
	Marital Status	Single
	Religion	Islam
	Political Affiliation	None
	Health Status	Good
Psychological Data	Stress Level	High
	Anxiety Level	Medium
	Depression Level	Low
	Self-Esteem	High
	Life Satisfaction	Medium
	Optimism	High
	Resilience	Medium
	Emotional Stability	High
	Personality Type	Extroverted
	Intelligence	High
Physical Data	Heart Rate	75
	Blood Pressure	120/80
	Cholesterol	180
	Blood Sugar	90
	Respiratory Rate	15
	Body Temperature	37.5
	Weight	70
	Height	1.75
	Body Mass Index	22.5
	Physical Activity	Low
Social Data	Family Size	4
	Family Income	Medium
	Family Structure	Nuclear
	Family Cohesion	High
	Family Support	High
	Family Communication	High
	Family Conflict	Low
	Family Stability	High
	Family Satisfaction	High
	Family Well-being	High